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VOLUME VIII 1949 NUMBER 2

THE USE OF THE AMIDOL TEST FOR DISSOLVED OXYGEN IN SEA WATER[1]

By

MAXIM LIEBER[2] AND ROLF ELIASSEN[3]

For many years the Winkler method with its various modifications has been the accepted procedure to determine the dissolved oxygen (D. O.) in fresh and saline waters (Amer. Public Health Ass., 1946). The accuracy of this volumetric test has proven itself in the laboratory where proper facilities and apparatus are available for such determinations. However, this test is too awkward and elaborate to be utilized in the field for sanitary and biological studies.

Although several attempts were made by Ramsay and Hofray (1901), Winkler (1911) and Rideal (see Discussion following Ramsay and Hofray, 1901) to utilize colorimetric procedures for a simplified D. O. field test, their efforts were not successful. Isaacs (1935) and Gilcreas (1935) introduced the use of Amidol (diaminophenol hydrochloride) as a colorimetric method for D. O. in fresh waters. At a pH of 5.1, produced by sodium citrate buffer, the D. O. would oxidize the Amidol at a reproducible rate. The oxidized Amidol in turn produces a polychrome reaction of yellow and red. The red shade of the reaction, when compared against the permanent Amidol standards formulated by Gilcreas (1935), proved sufficiently accurate to utilize this test for D. O. work in the field. When applied to brine or sea water, however, Isaacs (1935) and Gilcreas (1935) also found that,

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due to offshades in color and consequent high readings, this test as developed could not be utilized with any degree of accuracy.

This study is an attempt to develop a means whereby the simplicity and practicability of the Amidol test could be applied in marine activity.

PROCEDURE

The procedure in this study consisted essentially of conducting comparative tests on sea waters of varying salinities by both the Amidol and Winkler methods.

The details of this procedure were as follows.

Preparation of Waters of Varying Salinities. A variation in the salinity was obtained by mixing proportionate quantities of distilled water with filtered sea water. The final salinities of the prepared waters were determined by the procedure proposed by Service (1928). This salinity test is in reality a chlorinity test, since it was determined on an aliquot sample of the water with a standard solution of silver nitrate, with potassium chromate as an internal indicator. In conjunction with the salinity test, the specific gravity and temperature of the waters tested were obtained. The following are the various sea water dilutions prepared:

Approximate % dilution of sea water	*Salinity in parts per thousand by wt. of total dissolved solids*	*Temperature (°C.)*	*Specific gravity*
Zero	0.0	22.5	1.0000
10	3.3	22.5	1.0020
20	6.55	20	1.0050
30	9.8	19	1.0075
40	12.9	19	1.0095
50	16.1	17.5	1.0120
60	19.1	18	1.0150
70	22.35	16.5	1.0170
80	25.65	18.5	1.0200
90	28.95	14	1.0225
100	32.0	16	1.0250

Preparation of Waters of Varying D. O. Contents. Since it was necessary to prepare waters with varying D. O. contents, a method was devised to prepare water devoid of D. O. This latter water, mixed proportionately with an oxygen saturated water, produced solutions of varying and desired D. O. concentrations.

Fig. 1 is a diagrammatic sketch of the apparatus used for the preparation of deoxygenated water. A two-liter pyrex flask (E) was filled

with water containing the desired salinity. The water was boiled for approximately five to ten minutes, after which time the flame was turned off. The siphon tube from suction flask (D) was then inserted into and close to the bottom of flask (E). All the pinchclamps (1, 2, 3 and 4) were closed. The suction was turned on and pinchclamp No. 2

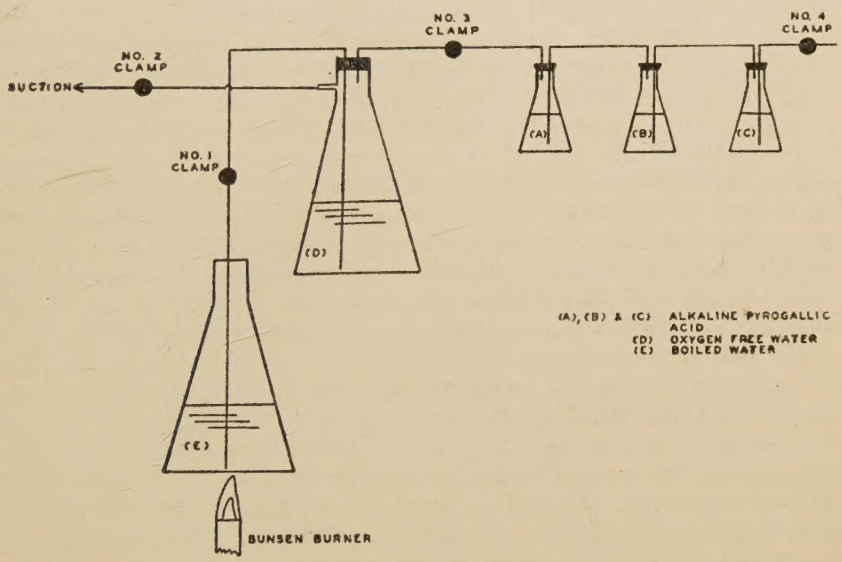

Figure 1. Diagrammatic sketch of apparatus used for the preparation of deoxygenated water

was opened slowly. This produced a partial vacuum in suction flask (D) which caused a back-siphonage of the water from flask (E) to flask (D). Pinchclamps Nos. 1 and 2 had to be controlled carefully, because the reduced pressure created a state of ebullition. After the desired amount of water was back-siphoned into suction flask (D), pinchclamp No. 1 was closed, the suction turned off and the deoxygenated water allowed to cool to room temperature under reduced pressure. This apparatus facilitated the removal of the oxygen gas dissolved in the original water by 1, increasing the temperature which decreased the solubility of oxygen in water, 2, utilizing suction to reduce the solubility of oxygen in water, and 3, boiling the water under reduced pressure and employing the scrubbing action of the ebullience induced in the system.

This apparatus produced waters with D. O. content of 0.2 p. p. m. as determined by the Winkler test. The deoxygenated water was

successfully stored in the apparatus under reduced pressure for several days without an appreciable increase in the D. O. concentration.

Color Comparison of Amidol Samples. The Amidol samples were compared with the permanent Amidol standards by use of a 15-watt General Electric fluorescent daylight lamp. The light shades in color in the low Amidol range (from zero to approximately 6.0 p. p. m. D. O.) were found simple to compare. However, it was found at concentrations greater than 6.0 p. p. m. D. O. that it was more difficult to compare this color. Therefore it is advisable for the novice to acquire experience in comparing the Amidol color at higher concentrations.

The Consolidation of Experimental Data. The tests were conducted on each of the prepared 11 dilutions of sea water. The Amidol tests were made according to the procedure outlined by Gilcreas (1935), with the comparison of the Amidol sample and standards taking place after the lapse of 30 minutes. On doing the Amidol test on the 10 and 20% sea waters it was noted in a few cases that a brownish offshade was present in the developed color. These Amidol samples were allowed to stand for an extended period, after which they were again compared with the standards. It was noted that the samples which originally possessed a brownish offshade, on standing approximately one and one half hours, approached the shade of the permanent Amidol standards. From that point, all Amidol tests were compared against the standards after the first 30 minutes, and then at 15 minute intervals until the lapse of two or three hours.

On examining the consolidated data at the completion of the tests on all the dilutions of sea water, it was discovered that wherever offshades occurred the colors blended back to the color range of the standards at the end of one and one-half hours. This increased the possibility of utilizing the Amidol test on sea water because it enabled the analyst to read a color which was no longer brownish but which compared favorably with the standards. Observations throughout the experiments indicated that the bulk of these offshades occurred in sea water with a D. O. concentration of approximately 2.0 through 6.5 p. p. m. The positive error also increased to a slight degree when the Amidol sample was allowed to stand one and one-half hours instead of only 30 minutes before comparison with the standards. However, this increase in error loses its significance, since the error occurs primarily in the higher D. O. range, which is the less critical range in biological and sanitary field activities. Table I contains only the significant data obtained from the Amidol tests after the lapse of one and one-half hours.

DISCUSSION

As previously stated, from the standpoint of accuracy the Winkler test is the accepted method for doing D. O. determinations. With that in mind it would be desirable to have any other method for testing D. O. approach as closely as possible the results of the Winkler test. If the Amidol test were as accurate as the Winkler test, the results of the Amidol test would always be equal to the results of the Winkler test for any specific concentration of D. O. A graphic representation of this ideal situation, with Amidol plotted as the ordinate and the Winkler as the abscissa, would result in a line which would have a 45° angle, and whose equation would be $X = Y$.

In actually comparing the Amidol tests with the Winkler tests it would be convenient to plot each of the Amidol results on a graph. These points would then be compared with the ideal line. As can be seen from the pertinent raw data, the results of the Amidol tests present a scatter of points on the graph which would be extremely difficult to interpret. To circumvent this difficulty it was decided that a more accurate and comparable statistical approach for the interpretation of the experimental data would be a necessary procedure.

Since the Amidol results present a scatter of points, the objective would be to obtain a curve which would best fit the data. When these points were placed in a graph it seemed evident that they clustered in a path which followed a linear trend. With this in mind it was necessary to obtain a straight line which would best fit these points and which could then be compared with the ideal line.. The formulas of these lines (Lines of Regression) for each of the experimental runs were obtained by the statistical procedure of the Least Squares Method. Since the trend of the data is linear, the resulting equations for the Lines of Regression are of the type

$$Y = a + bX .$$

To complete the statistical analysis, the Standard Error of Estimate (S_y) for each curve was calculated to determine whether the Lines of Regression calculated are the lines of best fit for the data.

$$S_y = \sqrt{\frac{\Sigma (d^2)}{N}},$$

S_y being the Standard Error of Estimate, d the deviation of the actual values of Amidol (Y) from the theoretical values of Amidol (Y_c) as derived from the Lines of Regression, or ($Y - Y_c$), and N the number of experiments conducted.

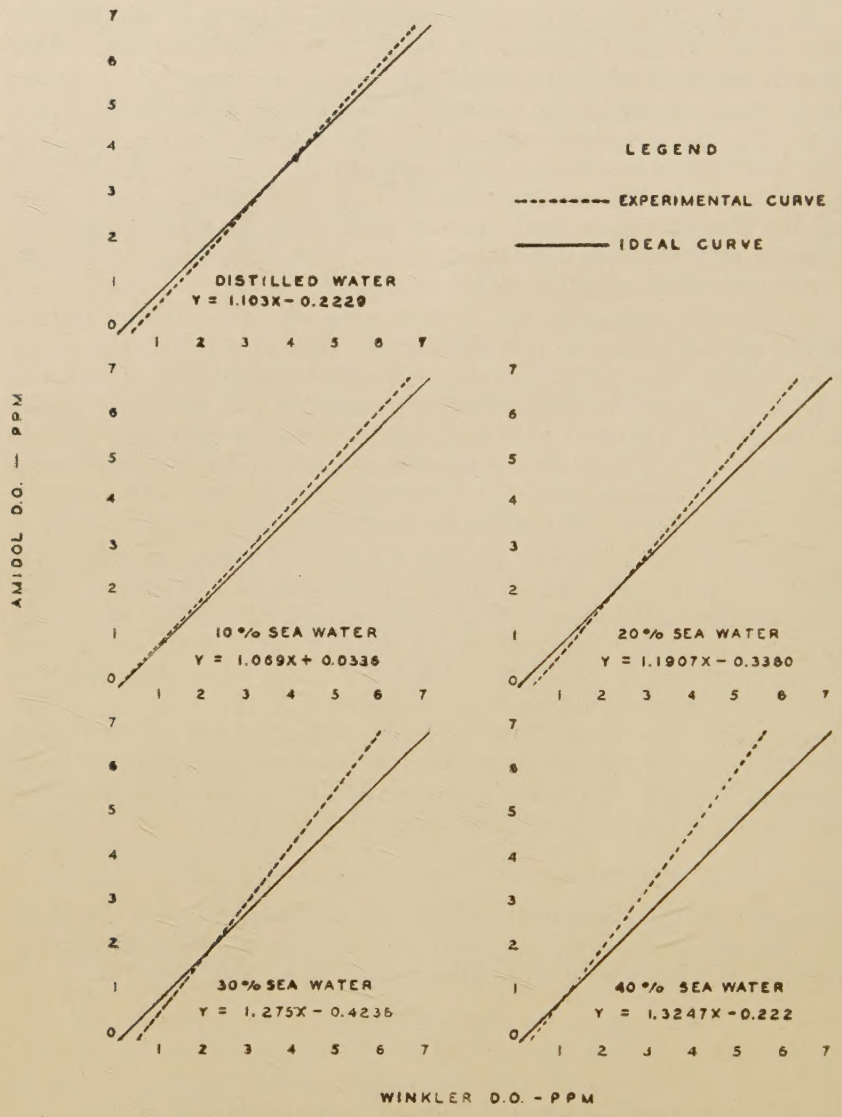

Figure 2. Graphic comparison of experimental results.

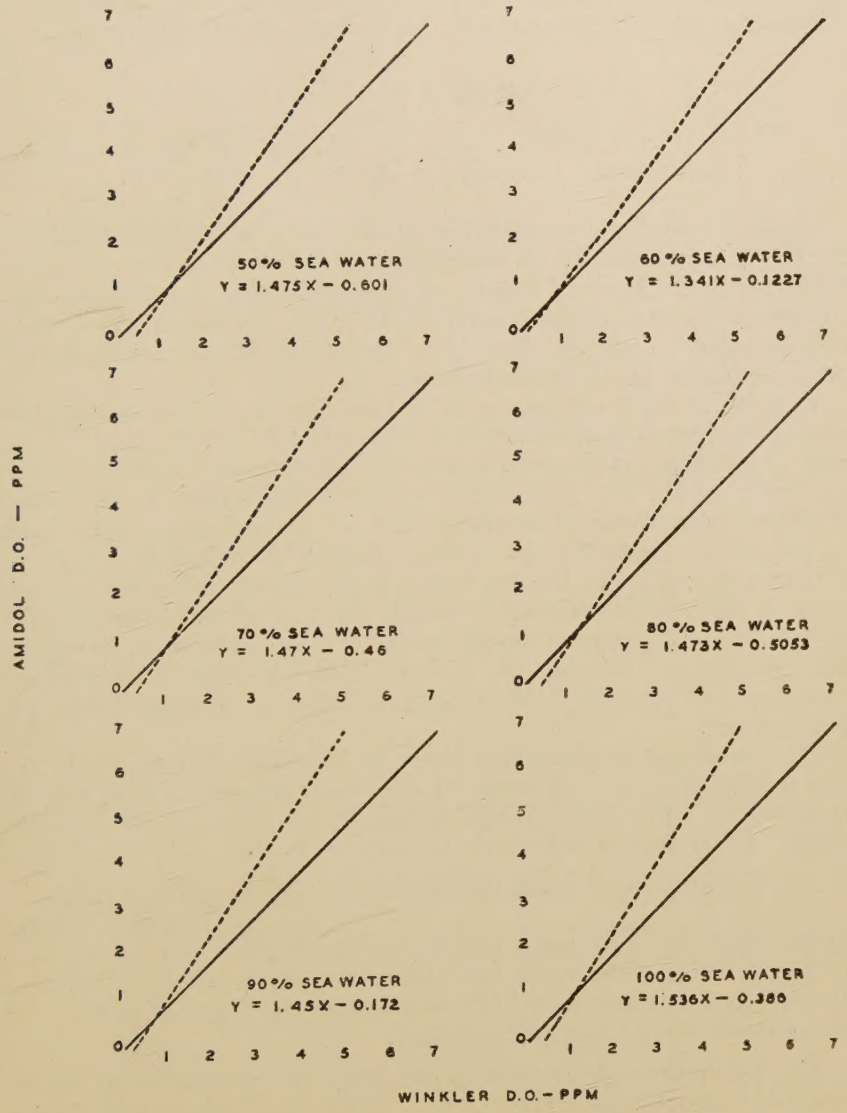
50% SEA WATER
Y = 1.475X − 0.601
60% SEA WATER
Y = 1.341X − 0.1227
70% SEA WATER
Y = 1.47X − 0.46
80% SEA WATER
Y = 1.473X − 0.5053
90% SEA WATER
Y = 1.45X − 0.172
100% SEA WATER
Y = 1.536X − 0.386
AMIDOL D.O. — PPM
WINKLER D.O. — PPM

Figure 3. Graphic comparison of experimental results.

In order to determine the degree of perfection in the relationship of X (Winkler) to Y (Amidol) in the above analysis, the Coefficient of Correlation (r) was calculated for each of the experimental runs.

$$r = \sqrt{1 - \frac{S_y^2}{\sigma_y^2}},$$

$$\sigma_y \text{ (Standard Deviation)} = \sqrt{\frac{\Sigma (Y)^2}{N} - \left(\frac{\Sigma Y}{N}\right)^2}.$$

Table II contains a consolidation of the complete statistical analysis for the data in Table I. A comparison of the coefficients of X in the formulas for the Lines of Regression show that the slope of these lines of best fit increase as the salinity of the water increases. Also, with this increase in salinity there is an increasing positive error in the range of higher D. O. concentrations.

As a visual aid in comparing these data, the Lines of Regression for each of the formulas were plotted in Figs. 2 and 3. These graphs show that all the Lines of Regression slope in such a manner that they tend to cluster around the "ideal curve" in the low significant D. O. range. This low range contains the critical D. O. levels which are so important to the sanitary engineer and aquatic biologist in their field activities. The accuracy that can be obtained on saline waters in the low critical range is sufficiently adequate to enable the Amidol test to be utilized in fish life and pollution studies on tidal bodies of water. It is of interest here to note that the Line of Regression for distilled water compares well with the Amidol results obtained by Gilcreas (1935) on fresh waters.

On examining the Standard Error of Estimate (S_y) in Table II, it is possible to predict a range within which all Amidol tests can be expected to fall. The average S_y for all the experimental runs is 0.348. From that we can conclude statistically that

a) 50 % of the cases would fall within 0.67 S_y = 0.23 p. p. m. (Probable Error),
b) 68 % of the cases would fall within one S_y = 0.35 p. p. m.,
c) 95 % of the cases would fall within two S_y = 0.70 p. p. m.,
d) 99.7% of the cases would fall within three S_y = 1.04 p. p. m.,
of their respective Lines of Regression.

The Coefficients of Correlation for these experiments as shown in Table II range from 98.69 to 99.65%. Since the Coefficients of Correlation are so close to 100%, they indicate that the S_y values are small and consequently that the determined Lines of Regression closely approach a perfect fit. This means that for each salinity the correla-

TABLE I. Comparative Results of the Winkler and Amidol Tests (p. p. m. D. O.)

Distilled Water		Sea Water																	
		10%		20%		30%		40%		50%		60%		70%		80%		90%		100%	
W.	A.	W.	A.	W.	A.	W.	A.	W.	A.	W.	A.	W.	A.	W.	A.	W.	A.	W.	A.	W.	A.
0.2	0.0	0.4	0.4	0.4	0.0	0.25	0.0	0.35	0.2	0.35	0.0	0.8	1.3	0.5	0.8	0.3	0.0	0.4	0.8	0.3	0.0
0.3	0.0	0.4	0.4	1.0	1.1	1.2	1.3	1.1	1.3	0.35	0.0	1.0	1.3	0.5	0.8	0.3	0.0	0.4	1.0	0.3	0.0
1.4	1.6	1.6	1.8	1.7	1.8	1.6	1.8	1.5	1.8	1.3	1.5	1.0	1.3	1.4	1.8	1.1	1.7	1.2	1.7	1.6	2.0
2.1	2.2	1.6	1.8	2.8	3.2	1.8	2.3	2.4	2.8	1.3	1.5	2.8	3.8	1.4	1.8	1.1	1.7	1.2	1.7	1.6	2.3
2.6	2.5	1.7	2.0	3.0	3.3	2.1	2.6	3.0	3.3	2.1	2.3	2.8	3.8	2.2	2.8	1.6	1.8	2.4	3.0	1.7	2.5
2.8	2.8	1.8	2.0	3.6	3.8	2.2	2.7	3.3	4.0	2.1	2.5	3.1	4.3	2.2	2.8	1.6	1.8	2.4	3.3	2.3	3.2
2.9	2.9	3.0	2.8	4.0	4.3	2.6	2.8	3.3	4.0	2.8	4.0	3.2	4.3	2.4	2.8	2.4	2.8	3.1	4.3	2.3	3.3
3.0	3.0	3.8	4.3	4.0	4.3	2.9	2.8	3.6	4.8	2.8	3.8	3.2	4.3	3.5	4.3	2.4	2.8	3.1	4.3	3.0	3.8
3.2	3.2	4.0	4.5	4.1	4.4	3.9	4.3	3.8	5.5	3.6	4.8	4.0	4.8	3.5	4.3	3.2	3.8	3.8	4.8	3.0	4.0
3.6	3.8	4.8	5.3	4.2	4.8	4.0	4.3	3.9	4.8	3.6	4.8	4.0	4.8	4.2	5.5	3.2	3.8	4.1	5.5	3.1	4.3
3.7	3.8	5.0	5.5	4.6	4.8	4.0	4.0	4.2	5.2	4.1	4.3	4.2	5.5	4.2	5.5	4.0	5.5	4.1	5.5	3.1	4.3
3.8	3.8	5.3	5.5	4.8	5.5	4.5	4.8	4.2	5.5	4.1	4.3	4.2	5.5	4.6	5.5	4.0	4.8	4.2	5.5	3.7	5.5
4.0	4.2	5.6	5.5	4.8	5.5	4.7	5.5	5.1	6.5	5.4	8.5	4.4	5.5	4.6	5.5	4.0	4.8	4.2	5.5	4.3	6.5
4.8	5.1	5.8	5.5	5.6	6.5	5.0	6.5	5.2	6.5	5.7	8.5	4.4	5.5	5.4	7.5	5.1	7.5	4.7	6.5	4.3	6.5
5.2	5.5	6.4	7.5	6.2	6.5	5.2	5.5	5.2	6.5	5.8	7.5	5.0	6.5	5.4	7.5	5.2	7.5	4.7	6.5	5.2	7.5
5.9	6.5	6.6	7.5	6.8	7.9	5.3	6.5	5.6	7.5	6.0	7.5	5.4	6.5	6.3	8.5	5.2	7.5	5.7	8.5	5.2	7.5
6.4	7.1	6.8	7.4	7.1	8.0	5.7	6.5	5.8	7.5	6.0	7.5	5.6	7.5	6.3	8.5	6.4	8.5	6.2	8.5	5.6	8.5
7.0	7.5	7.4	8.4	7.6	8.5	6.2	7.5	5.8	8.0	6.4	9.5	5.6	7.5	6.5	9.5	6.4	8.5	6.2	8.5	5.6	7.5
7.6	8.5	8.4	9.0	8.2	9.5	7.0	9.5	6.6	8.5	6.6	9.5	6.0	7.5	6.5	9.5	6.8	9.5	7.0	10.5	6.0	8.5
7.9	8.5	8.4	8.5	8.3	10.2	8.0	9.5	7.2	9.0	6.6	8.5	6.0	7.5	7.3	10.5	7.6	11.5	7.0	10.5	6.4	9.5
8.8	9.0	—	—	8.4	9.5	8.2	10.5	8.2	10.5	6.8	9.5	6.8	9.5	7.4	11.5	—	—	7.8	11.5	6.4	9.5
—	—	—	—	—	—	—	—	—	—	6.8	9.5	8.0	11.5	—	—	—	—	7.8	11.5	7.5	11.5
—	—	—	—	—	—	—	—	—	—	7.8	11.5	—	—	—	—	—	—	8.9	12.5	—	—
—	—	—	—	—	—	—	—	—	—	8.2	11.5	—	—	—	—	—	—	—	—	—	—

tion between Amidol and Winkler tests is high when expressed as a linear function as shown by the Lines of Regression. This high correlation makes it possible to determine what either X (Winkler) or Y (Amidol) would be when one of these is known.

TABLE II—Consolidation of Statistical Results

Water Tested	*Line of Regression*	*Standard Error of Estimate* (S_y)	*Coefficient of Correlation* (r)
Distilled Water	$Y = 1.103X - 0.2229$	0.176	99.25%
10% Sea Water	$Y = 1.069X + 0.0336$	0.334	99.25%
20% Sea Water	$Y = 1.191X - 0.3380$	0.230	99.65%
30% Sea Water	$Y = 1.275X - 0.4236$	0.430	98.79%
40% Sea Water	$Y = 1.325X - 0.2220$	0.262	99.50%
50% Sea Water	$Y = 1.475X - 0.6010$	0.560	98.69%
60% Sea Water	$Y = 1.341X - 0.1227$	0.348	98.99%
70% Sea Water	$Y = 1.470X - 0.4600$	0.442	99.05%
80% Sea Water	$Y = 1.473X - 0.5053$	0.367	99.35%
90% Sea Water	$Y = 1.450X - 0.1720$	0.342	99.50%
100% Sea Water	$Y = 1.536X - 0.3860$	0.258	99.65%
		Average 0.348	

TABLE III—Knudsen's (1901) Tables (Interpolations Made by the Sanitation Unit, New York State Conservation Dept.)

Specific gravity 60° F.	*Chlorides p. p. m.*	*Sea Water (100% = 17,500 p. p. m. Cl^-)* %
1.000	0	0
1.001	720	4
1.002	1440	8
1.003	2160	12
1.004	2880	16
1.005	3600	21
1.006	4320	25
1.007	5040	29
1.008	5760	33
1.009	6480	37
1.010	7200	41
1.011	7920	45
1.012	8640	49
1.013	9360	53
1.014	10080	57
1.015	10800	62
1.016	11520	66
1.017	12240	70
1.018	12960	74
1.019	13680	78
1.020	14400	82
1.021	15120	86
1.022	15840	90
1.023	16560	95
1.024	17280	99
1.0243	17500	100
1.025	18000	—

As shown previously, the use of the Amidol test on fresh or saline waters is sufficiently accurate in the low critical D. O. ranges to be valuable as a field test. Because of the high degree of association between the Winkler and Amidol tests, as shown by the high Coefficients of Correlation, it is now possible to utilize the Amidol test with a greater degree of accuracy in the high as well as in the low D. O. range. This can be accomplished as follows:

1. Determine the specific gravity of the water being tested when its temperature is approximately 60° F. This can be done with a urinometer whose specific gravity range is 1.000 to 1.025.
2. Convert the specific gravity of the water to the percentage sea water by using Knudsen's (1901) Tables (see Table III).
3. Do the Amidol test on the water according to the procedure outlined by Gilcreas (1935).
4. Compare the intensity of the color developed at the end of one and one-half hours with Gilcreas' permanent Amidol standards.
5. Utilize Figs. 2 and 3 to correct the Amidol D. O. to the true Winkler D. O. for the percentage sea water tested.

CONCLUSIONS

(a) Although the Amidol test on sea water is less accurate than the Winkler test, the former results compare favorably enough with the Winkler results to make the Amidol test satisfactory for D. O. determinations in field activities.

(b) The results of this test compare favorably with the Winkler test results in the low range of D. O. concentration.

(c) Additional accuracy with the Amidol test, both in the low and the high D. O. ranges, can be accomplished with the use of an hydrometer, Knudsen's Tables and the Lines of Regression for each salinity.

(d) This test can be of valuable assistance to sanitary engineers and marine biologists once they become accustomed to comparing the developed Amidol color.

(e) The equipment needed is not fragile and is economical, thus making this a practical field test.

ACKNOWLEDGMENT

The authors desire to express their sincere appreciation to M. H. Bidwell of the Sanitation Unit of the New York State Conservation Department for his co-operation and for making the facilities of his organization available to the first author.

APPENDIX

In order to facilitate the use of the Amidol test, the following summation is presented.

Preparation of Permanent Amidol Standards. According to Gilcreas (1935), the preparation of the permanent Amidol standards is made with cobalt chloride and potassium dichromate.

1. Stock Cobalt Chloride Solution: To 476 g. of cobalt chloride add approximately 800 ml. of distilled water. Dissolve the $CoCl_2 \cdot 6H_2O$, add 100 ml. of concentrated hydrochloric acid and make the volume up to one liter with distilled water. The solution may require filtration.

2. Stock Potassium Dichromate Solution: Dissolve 2.5 g. of $K_2Cr_2O_7$ and 20 ml. of concentrated hydrochloric acid in distilled water and make up to one liter.

TABLE IV. Gilcreas' Permanent Standards

p. p. m. Amidol D. O. Standard	*ml. Stock $CoCl_2$ Solution*	*ml. Stock $K_2Cr_2O_7$ Solution*
0.0	0.3	0.5
1.0	0.6	1.1
2.0	1.5	2.0
4.0	3.8	3.1
6.0	6.8	4.6
8.0	10.4	6.0
10.0	14.4	6.6
12.0	19.5	5.1
14.0	30.0	3.4

The accuracy of the Amidol test can be increased by utilizing a greater number of permanent Amidol standards within a desired D. O. range. This can be accomplished by plotting Gilcreas' data in Table IV which in turn will give the curves for the stock color solutions. To prepare a specific standard, the quantities of stock solutions required are read from the curves. These solutions, diluted to 50 ml. with distilled water and placed in a two-ounce French square bottle, will give the desired standard.

The Amidol Test. Reagents: a) Saturated solution of sodium citrate made by adding 750 g. of sodium citrate per liter of distilled water. b) Solid Amidol (diaminophenol hydrochloride).

Procedure: Collect sample according to the standard technique by the upward displacement of water in a two-ounce French square glass-stoppered bottle. Add 1.0 ml. of sodium citrate reagent, followed immediately by the addition of approximately 0.26 g. of solid Amidol. (Because of the staining effects of Amidol on skin, it has been found advisable to add the solid Amidol from the tip of a spatula.) Im-

mediately replace glass stopper in bottle to avoid the entrainment of air, mix sample well by inverting bottle several times and then let stand. After the lapse of 30 minutes for fresh water, or one and one-half hours for tidal waters, the sample is compared colorimetrically with the permanent Amidol standards.

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LABORATORY EXPERIMENTS ON WIND-GENERATED WAVES

By

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INTRODUCTION

It has long been known that the study of wind-generated waves on a water surface is difficult in the laboratory because of the large "fetch," or distance downwind, needed for the growth of waves large enough to behave as gravity waves rather than capillary ripples. Johnson (1948), on the evidence of experiments by Stanton et al. (1932) and those of his own at Clear Lake, California, concludes that a channel 311 feet long with a windspeed of 4.5 feet/sec. would reproduce the prototype conditions of a 10 mile fetch with a 40 m. p. h. wind. This assumes that one fetch and windspeed is a model of any other fetch, when the windspeeds vary according to the ordinary model law

$$\frac{T_1}{U_1} = \frac{T_2}{U_2},$$

where T is the period of the waves and U is the windspeed, the relation between Fetch/U^2 and T/U being given by an experimental curve that determines the minimum length of channel. However, in the example quoted, the prototype windspeed happens to be greater than a possible critical windspeed to which Munk (1947)[1] drew attention, while the model windspeed is less. If, as Munk suggests, the existence of this critical velocity corresponds to two different types of airflow over the waves, then the model law needs amplifying, for clearly it would not be justifiable to compare waves generated by one regime of airflow in a model with prototype waves generated by another. At still lower speeds there is another difficulty. For instance, referring to the other example quoted by Johnson (1948), where a 10 mile fetch with 5 m. p. h. wind is to be represented by a laboratory channel as long as 2,000 feet, then on the preceding basis the wind would be only 1.4 feet/sec., which is less than Jeffrey's (1925) minimum windspeed of 3.4 feet/sec., and no waves at all would be generated. Hence, indoor experiments

[1] However, additional experimental values of wind shear stress published by Bruch (1940) and Roll (1948), when plotted on the shear stress-windspeed axes in a similar manner to the evidence quoted by Munk, do not altogether confirm the existence of the critical velocity.

can be done only in channels of practical size when the windspeeds are greatly exaggerated, possibly even to the point at which they equal the prototype speeds, but even then the fetch and the dimensions of the waves must be restricted by some convenient artifice.

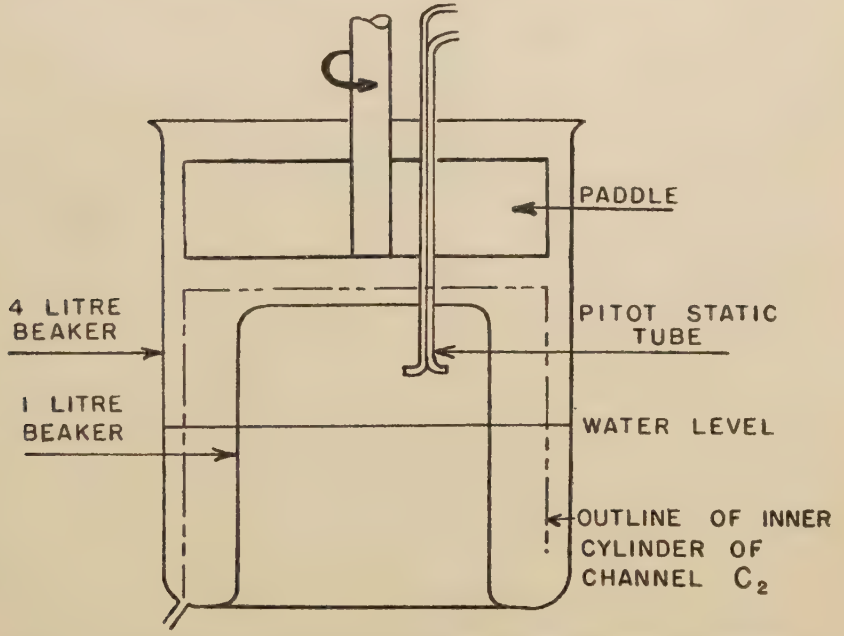

Figure 1. Channel C_1. Dotted lines show outline of tinplate island of Channel C_2.

Possibly some of the difficulties in studying the growth of waves may be avoided by an endless channel in the form of an annulus. If the wind is always acting circumferentially round the channel, then the waves should eventually become typical of those at an infinite fetch in a straight channel of the same cross section. The frictional effect of the wall remains, of course, and there is a possible disadvantage that the wavelength at any instant may be restricted to an integral submultiple of the length of the annulus; but the arrangement is compact, needs little power, and the shape and behavior of the waves are likely to be similar to those in Nature. The following experiments define the possibilities of such channels up to a size 6 feet in diameter and 1 H. P. input. The effect of varying the proportions of the annulus was investigated and the shape of the waves so formed was observed.

DESCRIPTION OF APPARATUS

Three sizes of channels, as shown in Figs. 1, 2 and 3, were used in the present experiments, and the leading dimensions are given in Table I.

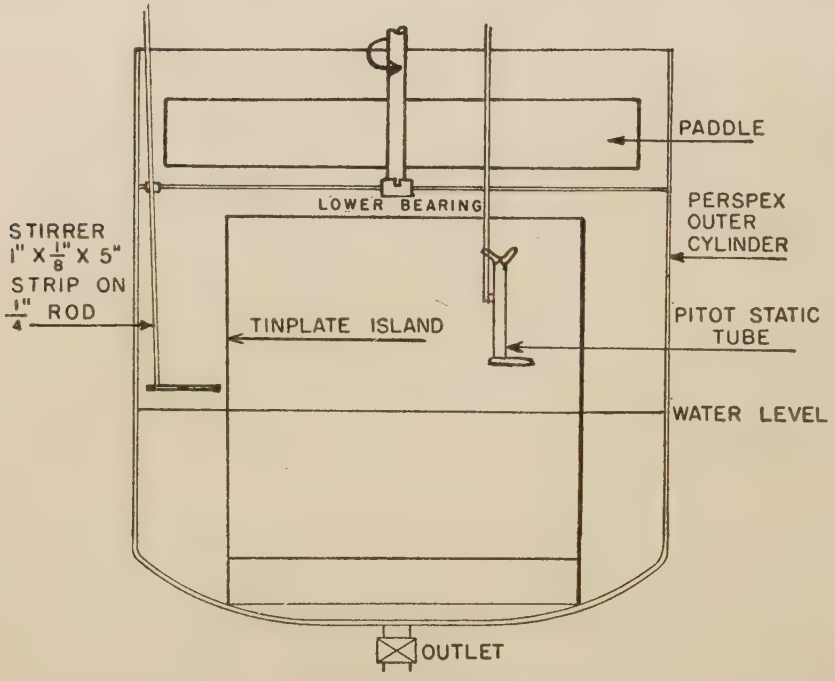

Figure 2. Channel B.

TABLE I. Measurements of Apparatus

Channel		*A*	*B*	C_1	C_2
Outer diameter	Inches	80	42	6	6
Inner diameter	Inches	72	30	4	5.3
Radial width	Inches	4	6	1	0.35
Outer dia./width		20	7	6	17
Depth from top of outer cylinder	Inches	12	40	8.7	8.7
Depth from top of inner or island cylinder	Inches	12	24.3	6.0	6.0
Depth of water	Inches	4.6	16	4	4
Height of pitot tube above still water level	Inches	2.6	3	1	1
Maximum power used	H. P.	1	½	¼	¼
Maximum speed of wind-making paddle	R. P. M.	120	370	5000	5000

Materials

Channel	*Outer Cylinder*	*Inner Cylinder*
A	Sheet steel with perspex window	Sheet steel
B	Perspex	Tinplate
C_1	4 litre glass beaker	1 litre glass beaker
C_2	4 litre glass beaker	Tinplate

The windmaker in the case of channel A consisted of a horizontal steel ring 6 feet in diameter and made of T-section bar, mounted coaxially with the channel and belt-driven by a 1 H. P. motor. Equally spaced round the ring were six flat plates 3½ by 4 inches, projecting 3 inches inside the rim of the channel and thus forming paddles to

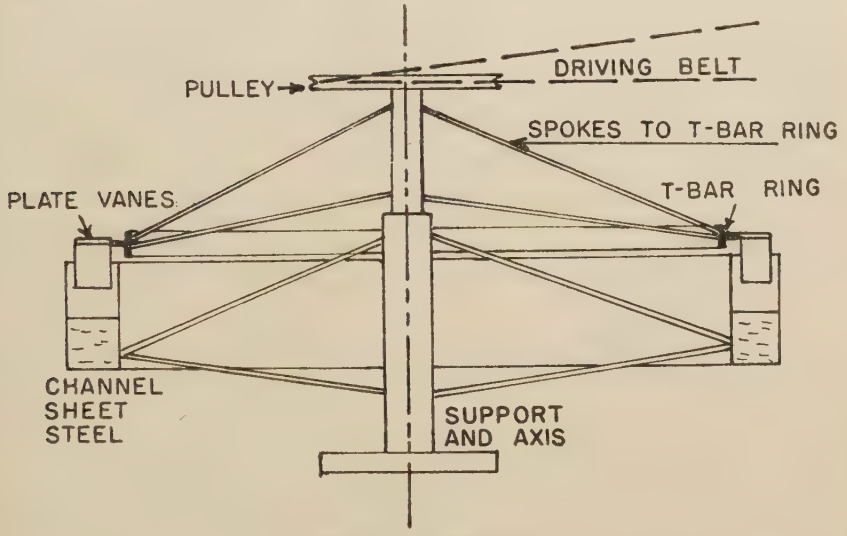

Figure 3. Channel A.

rotate the air within. In the other channels the inner "island" wall was lower than the outer wall. In the cylindrical space above the island there was a vertical shaft with a flat paddle which was rotated inside the outer cylinder by an electric motor, thereby swirling all of the air within the apparatus, including that in the annular channel.

The airspeed in the two largest channels was measured by Pitot tube connected to an inclined alcohol-manometer. For the smallest channel the only available Pitot-static tubes were too large, and these would have disturbed the air too much; therefore the static pressure was estimated from the reading of a brass tube of 0.03 inch bore facing downstream. The total pressure was measured by a similar tube facing upstream and soldered to the static tube. The arrangement when calibrated in a wind tunnel gave a pressure difference of 1.21 times the kinetic pressure.

OBSERVED WAVE-FORMS

Channels A *and* C_2. The waves made by gradually increasing the winds to various speeds in the several channels are summarized in Table II; in channels A and C_2, with diameter-to-width ratios of 20 : 1 and 17 : 1 respectively, the limiting velocities for the occurrence

TABLE II. Wave Forms and Limiting Speeds in Circular Wave Channels

Channel	Outer Dia Ins	O.Dia / Width	Wind speed (feet/second) 0	17	19	24·4	24·7	25·8	26	27·8
A	80	20	← NO WAVE PRODUCED OR MAINTAINED →							ROUGH TYPE WAVES →
B	42	7	NO WAVES →	WAVES PRODUCED ONLY IF WATER SURFACE IS DISTURBED → SMOOTH WAVES ONLY				← ROUGH WAVES →		WAVES APPEAR SPONTANEOUSLY
C	6	6	NO WAVES →	SMOOTH WAVES APPEAR SPONTANEOUSLY		← ROUGH WAVES →				
C_2	6	7	← NO WAVES PRODUCED OR MAINTAINED →						WAVES APPEAR SPONTANEOUSLY →	

of any waves at all were 27.8 feet/sec. and 25.8 feet/sec.; below these values, waves caused by a suitable stirrer soon died out and a glassy water surface remained. At and above the limiting velocity, waves appeared; in the large channel A they were about 27 inches long, averaging 2.7 inches high with a period of 0.75 seconds. Although they were not all of exactly the same shape or size, having small wind ripples superimposed upon the fundamentals, they were of typical trochoid appearance; in fact, they were complete replicas of sea waves and will be referred to subsequently as 'rough' waves. The steady state was reached by the time the initial disturbance had travelled 3 or 4 times round the annulus. The waves in channel C_2, appearing at 25.8 feet/sec., were naturally smaller, their wavelength being about ¾ inch and their height about 1/10 inch. They moved too fast for their shape to be seen precisely, but they seemed very regular and not quite sinusoidal. The windspeed could not be increased further since the fan motor was already well above its safe limit.

Channels B *and* C_1: *'Smooth' Waves.* In channels B and C_1, whose diameter-to-width ratios were 7 : 1 and 6 : 1 respectively, a more complicated wave system occurred. The minimum wind velocities to maintain any waves at all were then 17 feet/sec. and 19 feet/sec. respectively, and, at lower windspeeds the waves died out if temporarily generated by hand. In the small channel C_1, above the limiting windspeed, the waves appeared spontaneously and in a few seconds they spread all round the annulus. The waves, or rather ripples, appeared

perfectly smooth and nearly regular when viewed by light flashing 10 times a second. In the larger channel B, above the limiting airspeed and up to an airspeed of 26 feet/sec., waves, other than tiny ripples about ⅓ inch wavelength, did not appear spontaneously; however, when the airspeed was between the limiting speed and 24.7 feet/sec. and a disturbance was made with a stirrer, a group of low smooth waves or ripples formed and travelled round the annulus, leaving a plane mirror-like water surface before and after it. The leading waves in the group (the downwind ones) appeared nearly sinusoidal in shape and rather longer in wavelength, but the tail waves (the upwind ones) were asymmetrical, being very steep on their downwind (forward) sides and about ½ inch high and 4½ inches long from crest to crest. They resembled the cross section of sand dunes in the desert (see Bagnold, 1941: 202). The group of waves appeared to occupy a constant arc of the annulus for a given windspeed and initial agitation; then the higher the windspeed the larger was the arc occupied, until at about 22 feet/sec. the whole annulus had smooth waves in it.

The 'group' velocity of the smooth waves was measured and the results are given in Table III, the time for a revolution of the sharply defined upwind end of the train being taken.

TABLE III. Group Velocity of Smooth Waves

Windspeed (feet/sec. by Pitot)	*Group velocity (feet/sec.)*	*Group velocity ÷ Windspeed*
17.3	0.620	0.0359
17.8	0.632	0.0355
19.4	0.672	0.0347
20.6	0.725	0.0352
21.8	0.772	0.0354

It will be seen that the group velocity is a nearly constant proportion of the wind velocity.

Channels B *and* C_1; *'Rough' Waves.* In both of these channels there was a second limiting speed above which much larger "rough" waves appeared if the surface were agitated or if there were smooth-sided waves already existent. These second limiting airspeeds were measured as 24.7 feet/sec. for channel B and 24.4 feet/sec. for channel C_1. The waves in channel B at 26 feet/sec. were 3 inches high crest to trough, period 0.7 seconds, wavelength 27 inches; these values are near the theoretical maximum steepness. In a large proportion of waves the crests were not sharply defined, and so far as could be seen from outside the channel they had broken water on the downwind side. This may be the well known white cap phenomenon; but on the

small scale of the present experiments, and in the absence of the components of sea water which produce white froth, the effect is less visible.[2] The waves were similar to those produced in channel A.

These waves also appeared spontaneously from a plane surface when the windspeed exceeded 26 feet/sec., probably due to slight variations in windspeed; it was noticed sometimes that if the windspeed was between 24.7 feet/sec. and 26 feet/sec. rough type waves could be raised from a plane water surface if the fan was momentarily slowed down and then accelerated to its previous speed.

VERTICAL PROFILE OF WIND-SPEED

In both channels A and B, when no waves were present (i. e., windspeed below the second limiting value, and no disturbance made by stirrer), it was found that the windspeed at the midradius of the annulus was constant within $\pm$ 3% at all heights more than ¼ inch above the water surface. The same constancy occurred also when waves were present, with smooth ones and with rough ones, though the wind could not then be explored within 2 inches of the surface, because occasionally an extra large wave filled the Pitot with water. The constancy suggests that the wind gradient was determined mainly by the side walls and not by the water surface, and therefore the measurements do not define whether or not the water was acting aerodynamically as a smooth or as a rough surface.

EXPERIMENTS IN A CYLINDRICAL TANK

A few tests were made in a circular cylindrical tank without the island in the middle, the tank being the 42 inch diameter outer cylinder of channel B, the windmaker the one already described. The arrangement was thus equivalent to an annular channel with diameter/width ratio of 2.0, and with the air velocity approximately proportional to the radius. The airspeeds were measured 3 inches from the wall.

No waves or ripples were observed when the airspeed was less than 10.4 feet/sec. With windspeeds greater than this, however, a complicated three dimensional wave system developed which eventually resolved itself into that predicted by Rayleigh (1876). When the experiment was continued long enough the water surface again became plane although slightly disfigured by tiny wind ripples. The plane was not horizontal, however, but was sometimes steeply inclined,

[2] Some further experiments were done in channel A by using a solution of sea salt instead of the fresh water of other tests. The frothing on the breaking waves was much more noticeable, and it occurred immediately when the limiting windspeed was exceeded.

rotating round the vertical axis of the cylinder, this causing one crest and one trough per revolution. This is Rayleigh's first mode of vibration. Waves as much as 10 inches high from trough to crest at the circumference occurred in the 42 inch cylindrical tank with a windspeed of 20 feet/sec., corresponding to an inclination of the water-surface plane of 13°.

The same type of phenomenon occurred in the 6 inch diameter cylinder with a central "island" 2 inches in diameter, thereby forming a channel with an outer-diameter/width ratio of 3.0 No waves or ripples could be seen until the wind velocity was 10.4 feet/sec., measured at a point ½ inch from the outer circumference and 1 inch above water level. Then the same three-dimensional wave system formed as in the large tank.

AIRFLOW OVER WAVES; THE 'TROUGH EDDIES'

Munk (1947) tentatively suggested that smooth waves at subcritical windspeeds differ from rough waves by the lack of an eddy in the air lying in the trough between waves, although Jeffreys (1925) assumed that eddies exist even at the low windspeeds needed to create any waves. On the other hand, Roll (1948) deduced from vertical profiles of wind velocity (up to 29.2 feet/sec. at 2 m. height) that the water surface is aerodynamically smooth and that eddies are not present; he compares this result with that of Motzfeld (1937), who found that a solid wave-like surface with sharp crests was aerodynamically rough and had lee eddies.

An effort was made to investigate this point by using a smoke trail technique, but the rapid movement of the wave crests made visual observation uncertain. It is also difficult to choose a suitable smoke, that is, one which is sufficiently dense when injected into a 25 feet/sec. air stream but which contaminates neither water surface nor container and is not affected by moisture. One possible method is to use a small vertical jet which is at about still-water level, directed upwards. The jet is supplied with steam from an external boiler, and so long as it is covered by the water the steam is condensed. When a wave trough arrives the jet is uncovered and a puff of steam is shot out, thus forming a small white cloud of vapor. Subsequent observation of this cloud should show the direction of the airflow close to and relative to the wave, thereby disclosing the existence of an eddy. This was tried, but the steam condensed in the pipe leading to the jet and blocked it for the short time when the trough passed. Probably the difficulty could be overcome by more pressure and by the use of nonmetallic pipe.

A vertical jet of water which is fed steadily from a cistern external to the channel, and which rises to about ½ or ¾ wave height, was also tried with some success. When there was no wind relative to the channel the water rose and fell vertically, but any wind in one direction or another deflected it. The jet water was made visible with an acid

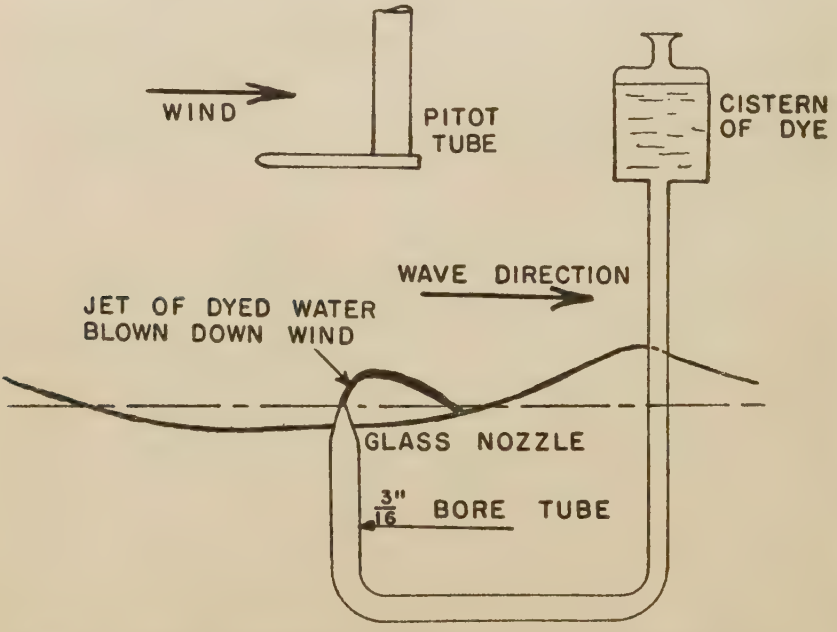

Figure 4. Detail of jet apparatus, to show absence of lee-eddy in trough of waves.

potassium permanganate solution which was bleached by the channel water having sodium sulphite in solution. The horizontal travel of the jet was calibrated in the channel against the windspeed by Pitot tube, the water level having been lowered sufficiently so that the jet was not washed over by the waves (see Fig. 4).

At all windspeeds, and with both 'smooth 'and 'rough' waves, the indicator jet was blown in the downwind direction with a deflection very little less than that in the free air stream. The greatest discrepancy was at the highest windspeed, with 'rough' type waves which averaged 3 inches from crest to trough, 27 inch wavelength, period 0.7 seconds. The waves were then travelling at about 3.4 feet/sec., their steepness ratio was 3/27 = 0.11, and they had the broken water at the crest as described previously (p. 125). The windspeed at 4

inches above still-water level was 25.8 feet/sec. and the jet was deflected in the wave troughs to a position corresponding to a windspeed of 21 feet/sec. If an eddy relative to the wave had been present, then the local windspeed reckoned relative to the tank would have been either reversed or less than 3.4 feet/sec.

Although experiments were not conclusive, they certainly did *not* support the much quoted lee-eddy picture. The present waves were very steep, almost approaching the maximum that is possible, while natural full scale waves are usually only half as steep and in that respect are correspondingly less likely to have lee-eddies. Perhaps the answer is to be found at the apex of the crest; if that is cusped, then the wind must separate and the lee-eddy can hardly be absent. In the model, surface tension prevents the cusp; in Nature, however, the waves, seldom tall enough to cusp of themselves, do so only when one wave overtakes another, and intermittent white-horses then appear, no doubt accompanied by intermittent lee-eddies. The whole spectrum of the wave frequencies seems involved, and perhaps the smaller waves are essential for the rapid growth of the larger ones, but the lee-eddy itself is not an essential part of wind-wave generation; the driving force remains whether or not the eddy is there, but the magnitude of the force is different, so that, without the eddies, stronger winds or greater distances are needed to build the waves.

DISCUSSION

Critical wind speeds. In all the present channels it appears that there is an abrupt change of state of the waves at some limiting velocity in the range 24–27 feet/sec. when the waves become rough and rippled. This is curiously near Munk's critical velocity of 6.6 m./sec. (21.7 feet/sec.), which he deduced by assuming a constant windspeed right down to the discontinuity at the water surface, a condition similar to that in these channels, where the velocity appears to be fixed by the side walls and where there is not a pronounced velocity gradient in the air above the water, as in Nature. Possibly the present windspeed values are not exact, since the Pitot tubes may have been sensitive to the curved flow; but the errors would be small, except possibly in channel C_2 where the airflow must have been disturbed by the 0.03 inch bore Pitot tube (0.075 inch outside diameter) that occupied a large part of the 0.35 inch wide annulus. Also the secondary currents in the air, causing a component velocity along the wave crests, may have played a part in the formation of the waves.

The apparent value of the limiting velocity seemed to increase somewhat with the diameter/width ratio of the channel, suggesting that side effects were exerting a measurable influence.

Lowest limiting windspeed. In all the channels, the minimum windspeed necessary to raise and maintain any waves at all is much higher than the value of 3.4 feet/sec. as found from observation over the sea. Inasmuch as the discrepancy is least for the low diameter/width ratio channels, it is probable that the surface tension and viscosity effects on the side walls are the cause, though the curving of the channel may have played a part, for the waves first generated by the wind are chiefly controlled by surface tension and tend to travel at constant speed, which is inconsistent with waves whose crests and troughs are radii to a circular channel.

Beginnings of waves. The evidence of the experiments in channel B, where an external agitation was required to produce waves when the wind was increased slowly and continuously to above the limiting speeds, may show that in Nature the tiny initial ripples are started by irregularities in the wind or by disturbances coming in as old waves and swell. Both 'wavy' and 'no wave' types of surface persisted for long periods at the same windspeed; but the 'no wave' surface changed immediately to the 'rough' type of wave when an external disturbance was given to the water.

ACKNOWLEDGMENTS

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SUMMARY

Experiments on the generation by wind of waves on a water surface have been made in channels of rectangular section, annular in plan, the wind being made by swirling the air above with a rotating paddle. The channels varied from 6 inches to 6 feet in diameter, the ratio of outer diameter to width up to 20, the input power up to 1 H. P. Some interesting wave forms were noticed, and there appeared to be a radical change of shape from smooth waves to wind-rippled large waves in the range 24–26 feet/sec., which is near Munk's proposed critical windspeed of 21.7 feet/sec. The minimum windspeed to produce any waves at all was much higher than that observed in Nature. With care, the windspeed could be gradually increased to 26 feet/sec. without any waves being formed; but the regime at that speed was unstable and waves were initiated by the slightest irregularity in the airflow.

The existence of lee eddies in the troughs of the waves was investigated by using a small vertical nozzle that projected a jet of water upwards when a trough passed. Although the waves were short, slow, and steep, there was no evidence of the existence of such eddies.

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MASS TRANSPORT IN OCEAN CURRENTS AND LATERAL MIXING

By

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1. *Equations of Mass Transport, with Lateral Mixing Taken into Account.* The problems of mass transport by ocean currents are no less important than are those of the currents themselves. Recently Sverdrup (1947) and Reid (1948) discussed the inter-relation between mass transport, pressure distribution and vertical mixing; they were successful in showing that the pressure distribution in the Pacific Equatorial Currents is being fairly maintained by the winds. In the present paper it is of some interest to discuss a certain relationship between mass transport and lateral mixing in a rotating enclosed sea.

It is natural to consider that lateral mixing in a current takes place mainly at right angles to the stream. Thus the frictional terms in the dynamic equations will be given by

$$\frac{\partial}{\partial y}\left(A_l\frac{\partial u}{\partial y}\right)+\frac{\partial}{\partial z}\left(A_z\frac{\partial u}{\partial z}\right) \text{ and } \frac{\partial}{\partial x}\left(A_l\frac{\partial v}{\partial x}\right)+\frac{\partial}{\partial z}\left(A_z\frac{\partial v}{\partial z}\right)$$

respectively (Sverdrup et al., 1942: 475), A_l and A_z being the coefficients of horizontal and vertical eddy viscosity respectively, u and v the horizontal velocity components parallel to the x- and y-axis respectively (the x-axis being chosen positive eastward, the y-axis at right angles *contra solem* to it, with the z-axis positive vertically downward; hence, in the northern hemisphere the y-axis is taken positive northward).

If we assume a stationary state and neglect the nonlinear terms, the dynamic equations become

$$\left.\begin{aligned}\frac{\partial}{\partial y}\left(A_l\frac{\partial u}{\partial y}\right)+\frac{\partial}{\partial z}\left(A_z\frac{\partial u}{\partial z}\right)+\lambda\rho v-\frac{\partial p}{\partial x}=0\\ \text{and } \frac{\partial}{\partial x}\left(A_l\frac{\partial v}{\partial x}\right)+\frac{\partial}{\partial z}\left(A_z\frac{\partial v}{\partial z}\right)-\lambda\rho u-\frac{\partial p}{\partial y}=0,\end{aligned}\right\} \quad (1)$$

where p is the pressure, ρ the density of the water, and λ the Coriolis parameter.

Integrating both sides of the equations in (1) from the surface to a depth d at which the baroclinic distortion can be considered small, and assuming A_l to be independent of z, we obtain

$$\left.\begin{aligned} \frac{1}{\rho}\frac{\partial}{\partial y}\left(A_l\frac{\partial M_x}{\partial y}\right)+\tau_x+\lambda M_y-\left(\rho g d\frac{\partial \zeta}{\partial x}+\frac{\partial P}{\partial x}\right)=0\,; \\ \frac{1}{\rho}\frac{\partial}{\partial x}\left(A_l\frac{\partial M_y}{\partial x}\right)+\tau_y-\lambda M_x-\left(\rho g d\frac{\partial \zeta}{\partial y}+\frac{\partial P}{\partial y}\right)=0\,. \end{aligned}\right\} \tag{2}$$

In these equations the pressure integral is denoted by P and the sea surface by $-z=\zeta\,(x, y)$ instead of $z=0$, since, due to certain factors such as the piling up of water by wind action, variations in density from place to place, etc., it does not necessarily coincide with any of the geopotential surfaces. Thus $\frac{\partial \zeta}{\partial x}$ and $\frac{\partial \zeta}{\partial y}$ give the geopotential slope of the sea surface.

Now we may consider that the mass transports M_x and M_y vanish along and across a solid boundary in the presence of lateral mixing; this will be rigidly true if the sea is enclosed by a vertical wall down to at least the depth d.

A third equation connecting M_x and M_y can be derived from that of continuity

$$\frac{\partial u}{\partial x}+\frac{\partial v}{\partial y}+\frac{\partial w}{\partial z}=0\,.$$

Integrating this equation from the surface to the level d, and assuming that the vertical component w is so small that it can be neglected at level d, we obtain

$$\frac{\partial M_x}{\partial x}+\frac{\partial M_y}{\partial y}=0\,. \tag{3}$$

Equation (3) enables us to introduce a function $\psi\,(x, y)$, such that

$$M_x=\frac{\partial \psi}{\partial y}\,;\quad M_y=-\frac{\partial \psi}{\partial x}, \tag{4}$$

which is similar to the current function and which may be called tentatively "transport function." Using this function, (2) may be written

$$\left.\begin{aligned} \frac{1}{\rho}\frac{\partial}{\partial y}\left(A_l\frac{\partial^2 \psi}{\partial y^2}\right)-\lambda\frac{\partial \psi}{\partial x}+\left(\tau_x-\rho g d\frac{\partial \zeta}{\partial x}-\frac{\partial P}{\partial x}\right)=0\,; \\ -\frac{1}{\rho}\frac{\partial}{\partial x}\left(A_l\frac{\partial^2 \psi}{\partial x^2}\right)-\lambda\frac{\partial \psi}{\partial y}+\left(\tau_y-\rho g d\frac{\partial \zeta}{\partial y}-\frac{\partial P}{\partial y}\right)=0\,, \end{aligned}\right\} \tag{5}$$

and on the condition that transport vanishes across and along the boundaries,

$$\left(\frac{\partial \psi}{\partial x}\right)_C = \left(\frac{\partial \psi}{\partial y}\right)_C = 0\,, \tag{6}$$

when the curve C represents the coastline.

2. *Transformation into a Variational Problem.* The problem represented by (5) and (6) can now be shown to be equivalent to a variational problem in which we should make a minimum the integral $I(\psi)$ defined as

$$I(\psi) = \iint_{(C)} \left\{ \frac{A_l}{2\rho}\left(\frac{\partial^2 \psi}{\partial x^2}\right)^2 - \frac{A_l}{2\rho}\left(\frac{\partial^2 \psi}{\partial y^2}\right) - \lambda \frac{\partial \psi}{\partial x}\frac{\partial \psi}{\partial y} + \left(\tau_x - \rho g d \frac{\partial \zeta}{\partial x} - \frac{\partial P}{\partial x}\right)\frac{\partial \psi}{\partial y} + \left(\tau_y - \rho g d \frac{\partial \zeta}{\partial y} - \frac{\partial P}{\partial y}\right)\frac{\partial \psi}{\partial x} \right\} dx dy\,, \tag{7}$$

where the integration should be carried out over the entire water surface surrounded by the curve C.

3. *Solution of the Problem in an Enclosed Sea.* Now let $\chi_i(x, y)$ $(i = 1, 2, \ldots, s)$ be a group of functions that are linearly independent of one another and that satisfy the same conditions along the boundaries as $\psi(x, y)$. Suppose, then, that we have

$$\psi(x, y) = \sum_i A_i \chi_i(x, y), \tag{8}$$

where $A_1, A_2, \ldots, A_s$ are constants to be determined.

Substituting (8) in (7), and making $\delta I = 0$, or

$$\frac{\partial I}{\partial A_1} = 0\,, \frac{\partial I}{\partial A_2} = 0\,, \ldots\,, \frac{\partial I}{\partial A_s} = 0\,,$$

we have the s linear simultaneous equations

$$\sum_i A_j E_{ij} = G_i, \quad (i, j = 1, 2, \ldots, s) \tag{9}$$

which determine the constants, where

$$\left.\begin{aligned} E_{ij} = &\iint_{(C)} \frac{A_l(\xi, \eta)}{\rho} \cdot \frac{\partial^2 \chi_i}{\partial \xi^2} \cdot \frac{\partial^2 \chi_j}{\partial \xi^2} \cdot d\xi \cdot d\eta \\ &- \iint_{(C)} \frac{A_l(\xi, \eta)}{\rho} \cdot \frac{\partial^2 \chi_i}{\partial \eta^2} \cdot \frac{\partial^2 \chi_j}{\partial \eta^2} \cdot d\xi \cdot d\eta \\ &- \iint_{(C)} \lambda(\eta) \cdot \frac{\partial \chi_i}{\partial \xi} \cdot \frac{\partial \chi_j}{\partial \eta} \cdot d\xi \cdot d\eta \\ &- \iint_{(C)} \lambda(\eta) \cdot \frac{\partial \chi_i}{\partial \eta} \cdot \frac{\partial \chi_j}{\partial \xi} \cdot d\xi \cdot d\eta \end{aligned}\right\} \tag{10}$$

$$\left.\begin{aligned} G_i = &- \iint_{(C)} \left(\tau_x - \rho g d \frac{\partial \zeta}{\partial \xi} - \frac{\partial P}{\partial \xi} \right) \frac{\partial \chi_i}{\partial \eta} \cdot d\xi \cdot d\eta \\ &- \iint_{(C)} \left(\tau_y - \rho g d \frac{\partial \zeta}{\partial \eta} - \frac{\partial P}{\partial \eta} \right) \frac{\partial \chi_i}{\partial \xi} \cdot d\xi \cdot d\eta \end{aligned} \right\} \tag{11}$$

The practical evaluation of E_{ij} and G_i is not difficult if we have the information as to the analytic form of $A_l\ (x, y)$. Of course the Coriolis parameter varies in the meridional direction, which should be taken into consideration. Many interesting results might be expected by assuming the dependence of λ on y (Stommel, 1948).

4. *Choice of the Functions* $\chi_s\ (x, y)$. The group of functions which satisfy the same boundary conditions as do $\psi\ (x, y)$ can be constructed without difficulty for any shape of the coastline.

Suppose $F\ (x, y) = 0$ gives the curve C. Then it is easily seen that we should take

$$\chi_s\ (x, y) = \{F\ (x, y)\}^2\ K_s\ (x, y),$$

where $K_s\ (x, y)$ are all linearly independent.

For a rectangular basin bounded by

$$x = \pm a \text{ and } y = \pm b,$$

we may take

$$\chi_s\ (x, y) = M_m\ (x) \cdot M_n\ (y)\ ,$$

where

$$\begin{aligned} M_m\ (x) &= \cos \frac{m\pi x}{2a} \cos \frac{\pi x}{2a} && \text{for } m \text{ odd,} \\ &= \sin \frac{m\pi x}{2a} \cos \frac{\pi x}{2a} && \text{for } m \text{ even,} \end{aligned}$$

and

$$\begin{aligned} M_n\ (y) &= \cos \frac{n\pi y}{2b} \cos \frac{\pi y}{2b} && \text{for } n \text{ odd,} \\ &= \sin \frac{n\pi y}{2b} \cos \frac{\pi y}{2b} && \text{for } n \text{ even.} \end{aligned}$$

5. *Concluding Remarks.* In this paper is given a theoretical model of water movement in an enclosed rotating sea induced by wind under the action of lateral mixing and corresponding to a given mass field. While this consideration is purely theoretical, practical application is promising. Efforts in this direction are now being continued.

Finally, the author wishes to express his best thanks to Walter H. Munk and Robert O. Reid of the Scripps Institution of Oceanography and to Yngve H. Olsen of the Sears Foundation for their generous assistance and advice.

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COMPARATIVE BIOCHEMICAL STUDIES ON THE LIPIDS OF MARINE INVERTEBRATES, WITH SPECIAL REFERENCE TO THE STEROLS[1]

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INTRODUCTION

For a number of years the author and his collaborators have been engaged in a systematic comparative study of the fatty constituents of marine invertebrates. So far special attention has been devoted mainly to the unsaponifiable fractions of such fats, in particular to the sterols which they contain. Work on the phosphatide fractions of invertebrate fats and their saponifiable components is now in progress and will be discussed in a future publication.

The British biochemist Dorée must receive credit for the first significant comparative study on the occurrence of sterols in animals. The report of his observations, published in 1909 (36), represents an

[1] Contributions to the study of Marine Products, XXV.

outstanding contribution to the general subject of comparative biochemistry, but it has not yet received the attention it justly deserves. The object of his studies has been expressed with admirable clarity in the introductory paragraph of his report.

> If cholesterol is a body which is one of the primary constituents of animal protoplasm, we should expect to find it not only in the highly organized animals, but throughout the series from Chordata to Protozoa; or if cholesterol itself were not present its place should be filled by other and closely related forms. In the latter case it might be found that each of the great classes of the animal kingdom was characterized by the presence of a different member of the cholesterol family. On the other hand, if cholesterol is not of primary importance to all forms of life, it is not impossible that animals might be found into the composition of whose protoplasm it did not enter.

Dorée isolated and characterized the sterols from one or two representatives of most of the important phyla. In establishing the presence of sterols in all the animals under investigation he therefore inferred that sterols were typical constituents of all animals. Cholesterol was shown to be present not only in all warm-blooded animals but also in several of the invertebrates. Although Dorée did not find a typical sterol for each of the phyla, he supplied convincing evidence that at least two marine invertebrates, the sponge, *Cliona celata*, and the starfish, *Asterias rubens*, contain sterols distinctly different from cholesterol. About the same time the occurrence of sterols other than cholesterol had been demonstrated in sponges by Henze (46) and in insects by Menozzi and Moreschi (70).

For almost 25 years following the publication of Dorée's paper comparative studies on the occurrence of sterols in animals remained dormant. Numerous articles have appeared during that period which deal with the isolation of sterols from vertebrates, their principal value resting on the demonstration of the ubiquity of cholesterol in this phylum. However, little if any attention was paid to the sterols of marine invertebrates, which, on the basis of earlier observations, appeared to be the most interesting. With the elucidation of the structure of cholesterol in 1932 and the subsequent phenomenal development of steroid chemistry, the general interest in this group of compounds underwent substantial expansion. The time had become ripe for a continuation and expansion of Dorée's work which might lead eventually to an understanding of the biochemical evolution of the sterols. It is because of their great abundance and diversity that the marine invertebrates have so far become the almost exclusive subjects for such a comparative study.

Only a few of the various types of naturally occurring organic compounds are as well suited for a comparative investigation as the sterols. They are apparently present in all animals and in a diversity adequate for comparative purposes. In addition they enjoy great stability and they may be isolated readily and determined quantitatively. The same holds true in a somewhat lesser degree for other components of the unsaponifiable fraction of fats.

With the exception of 7-dehydrosterols of the type of Provitamin-D, the sterols are remarkably resistant against the action of heat and air, particularly when mixed with other organic matter. Such stability permits the investigator to accumulate and store substantial quantities of biological material, air-dried or otherwise preserved, without fear of significant alterations of the sterol present in it. It also makes possible the utilization of old, dried museum specimens. In several instances it has been shown that samples of dried sponges more than 50 years old did not differ noticeably in their sterol content from freshly collected specimens.

METHODS

The isolation of the sterols and other unsaponifiable matter is easily accomplished as a rule, and it is only in rare instances that complications may be expected. When air- or vacuum-dried material is available, it is best broken into small pieces and then thoroughly extracted with acetone in a suitable Soxhlet extractor. For the extraction of quantities exceeding several hundred grams, the type of extractor shown in Fig. 1 has been found to be most effective. It forces the vapors of the solvent to pass through the center of the extraction bulb, thereby heating the solvent in contact with the material and accomplishing a hot extraction. As the extraction proceeds the sterol occasionally begins to separate from the solvent in the lower flask. After from one to two days of continuous extraction, depending on the amount of material, the extract is evaporated to dryness.

The residue is then refluxed with benzene in an apparatus (Fig. 2) which permits, by codistillation, the steady removal of any water contained in the residue. Once this process is completed, the benzene solution is decanted off some brown, smeary material which adheres to the wall of the flask. The flask is then rinsed several times with fresh benzene. The combined benzene extracts are evaporated to dryness, and the residue is dried to constant weight at about 100° C.

In this communication it is a residue thus obtained which will be referred to as the fat.

When fresh wet material is to be extracted it is best minced and then refluxed with benzene in a round bottom flask of suitable size. As before, the water is removed continuously by codistillation with

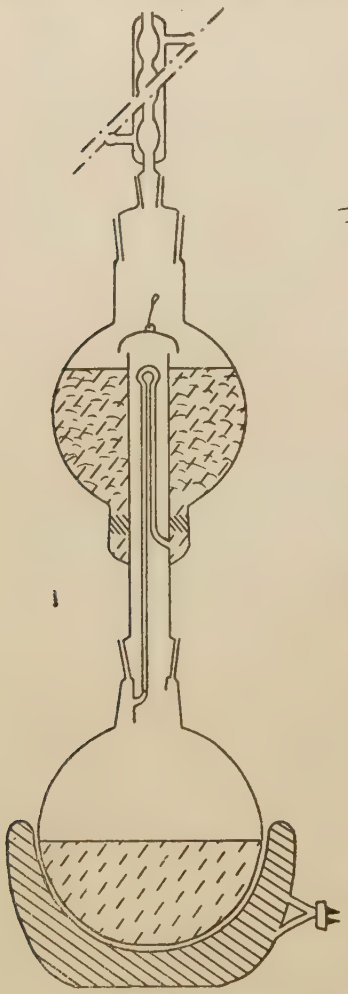


Figure 1. Large-scale extractor.

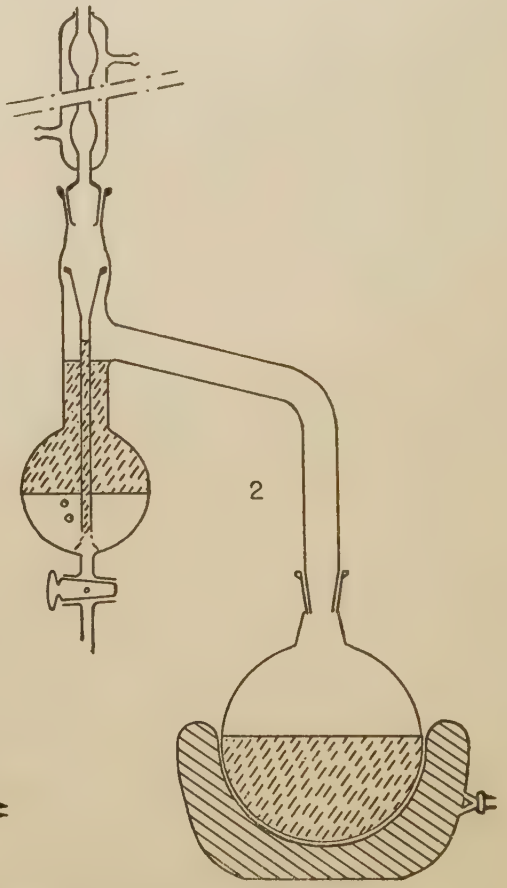


Figure 2. Benzene dehydrator.

benzene. In this manner several liters of water may be removed from wet material within 24 hours. As soon as no more water is carried over by the benzene, the extract is decanted from the residue, which is then thoroughly extracted with benzene in a Soxhlet apparatus.

All benzene extracts are eventually combined and evaporated to dryness as before.

In order to obtain the unsaponifiable material, one part by weight of fat is refluxed for one hour with ten parts by volume of a 10% solution of potassium hydroxide in 75% ethanol. The solution is then cooled and mixed with two volumes of water. The mixture is extracted with several portions of ether until the last remains colorless, or practically so. The ether extracts are combined, washed several times with water and evaporated to dryness. The water adhering to the residue is removed by codistillation with benzene. The consistency of the residue at room temperature ranges from a viscous oil to a high melting solid, depending on the amount of sterol present in the mixture.

In many instances the bulk of the sterol may be obtained from the total unsaponifiable fraction by simple recrystallization. This is best accomplished by refluxing the unsaponifiable material with successive portions of methanol until all but a small amount of brown material has become dissolved. Cooling of the combined methanol extracts leads to the separation of the crystalline sterol or sterol mixture. The amount of sterol remaining in the mother liquor is then determined quantitatively by carrying out a precipitation with digitonin on an aliquot part.

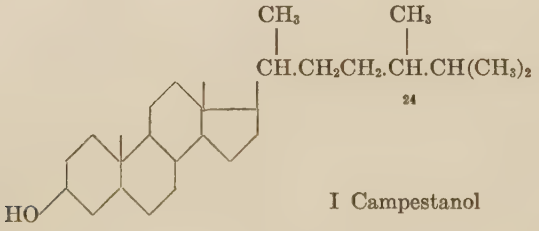
The unsaponifiable fraction of certain coelenterates contains the sterols mixed with such substantial quantities of higher aliphatic alcohols and hydrocarbons as to make separation by recrystallization difficult. In such cases, and particularly where only small amounts are available, it is advisable to precipitate the total sterol with digitonin and to recover it from the digitonide by Bergmann's method (10). The separation of nonsteroid alcohols and of hydrocarbons is best accomplished by way of the alcohol sulfates (9).

STEROLS FROM ANIMALS

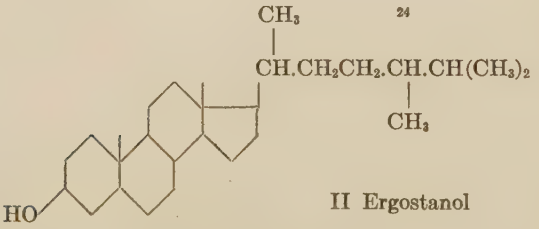
Prior to the beginning of the present studies the only known major animal sterol was the well studied cholesterol. This sterol is frequently accompanied by small amounts of its 5, 6-dihydro- and 7-dehydro-derivative. In addition, the existence of other animal sterols had been indicated in certain sponges, echinoderms and insects. At present the structures of eight new animal sterols have been established and about six more have been isolated, the structures of which remain uncertain thus far.

The animal sterols known at present may be divided into three groups according to the number of carbon atoms they possess. The most prominent member of the group, with 27 carbon atoms, is the

well known cholesterol, $C_{27}H_{46}O$. Other sterols are of the order C_{28} and C_{29}. These may be regarded as derivatives of corresponding C_{27}-sterols with a methyl or an ethyl group attached to the C-24-atom of the side chain. The presence of such groups at this carbon atom confers asymmetry upon it and hence makes possible the existence of two isomers, epimeric at C-24. To clarify this point it is convenient to write the structure of one such C-24-methyl epimer, with the methyl group above the side chain as in formula I, and to refer to it as a C-24-a-methyl sterol. Correspondingly, the C-24-b-methyl epimer would be represented as shown by formula II. In the case of



I Campestanol



II Ergostanol

the saturated C-24-methyl sterols, campestanol has been named the a- and ergostanol the b-methyl derivative of cholestanol; these two compounds are now being used as reference substances. By a series of deductions it has been shown that stigmastanol is the corresponding C-24-b-ethyl and poriferastanol the corresponding C-24-a-ethyl derivative[2] (25).

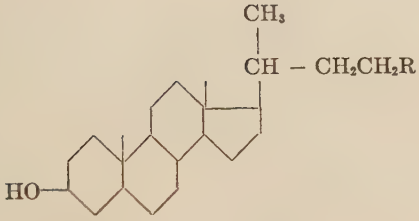
Table I presents a survey of the known types of sterols which have been isolated so far from marine invertebrates and certain other animals. In addition it shows which sterols, or their C-24-epimers, have also been found to occur in plants. In the case of the C-24-methyl and ethyl derivatives it is of interest to note that the representatives of the a-series predominate in marine animals and those of the

[2] The numbering system originally proposed by Bergmann and Low (25) has been changed to conform with that suggested by Fieser and Fieser (39).

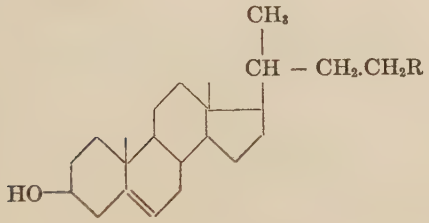
TABLE I.—Naturally Occurring Sterols

A. Cholestane-Series

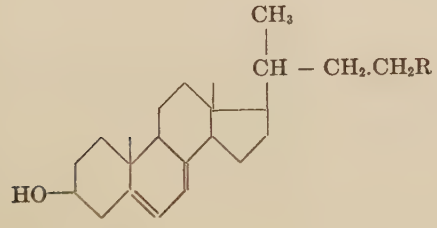
R = $-CH_2.CH(CH_3)_2$



Cholestanol (Dihydrocholesterol)
(Sponges)



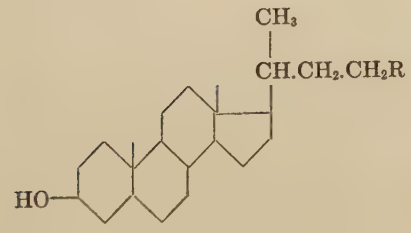
Cholesterol
(Animals)



7-Dehydrocholesterol
(animals, small amounts)

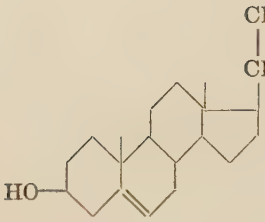
B. 24-Methyl-Cholestane Series

a-series: R = $-CH(CH_3).CH(CH_3)_2$; b-series: R = $-CH(CH_3).CH(CH_3)_2$ (with CH_3 on the first CH)



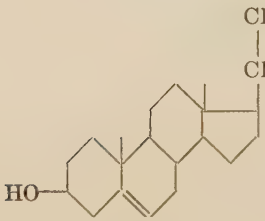
a-series: Campestanol
b-series: Ergostanol

TABLE I.—*Continued*



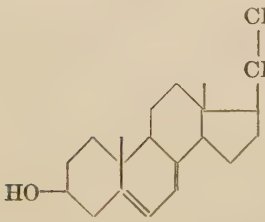
a-series: Campesterol
(Plants, Sponges, Mollusks?)

b-series: Dihydrobrassicasterol
(Plants, Sponges?)



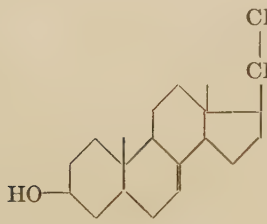
a-series: Chalinasterol (Ostreasterol)
(Mollusks, Sponges, Coelenterates)

b-series: Brassicasterol (Shakosterol)
(Plants, Mollusks)



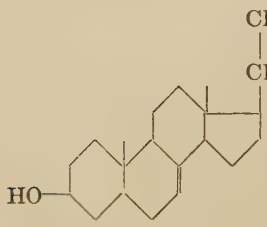
a-series: Unknown

b-series: Ergosterol
(Fungi, Annelids)



a-series: Stellastenol
(Echinoderms)

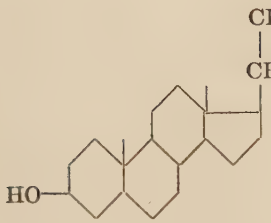
b-series: γ-Ergostenol
(Fungi)



a-series: Stellasterol
(Echinoderms)

b-series: Dihydroergosterol
(Fungi)

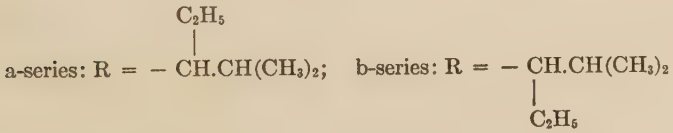
TABLE I.—*Continued*

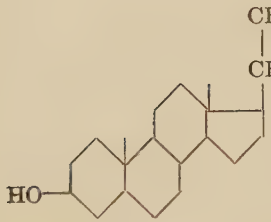


a-series: Neospongosterol
(Sponges)

b-series: Unknown

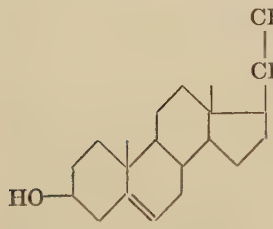
C. 24-Ethyl-Cholestane Series.





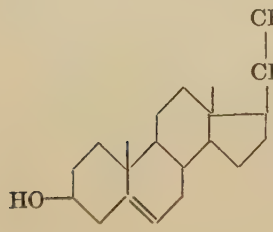
a-series: Poriferastanol (γ-Sitostanol)
(Plants, Sponges)

b-series: Stigmastanol
(Plants)



a-series: Clionasterol (γ-Sitosterol)
(Marine Invertebrates, Plants)

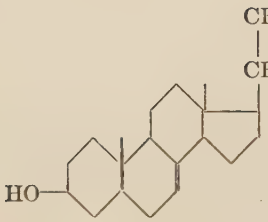
b-series: Dihydrostigmasterol
(Plants)



a-series: Poriferasterol
(Sponges)

b-series: Stigmasterol
(Plants)

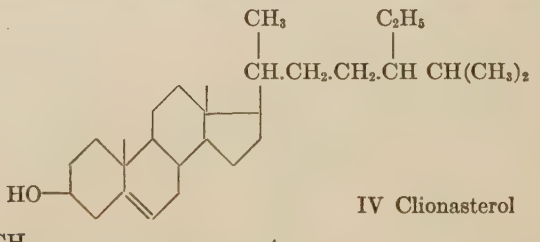
TABLE 1.—*Continued*



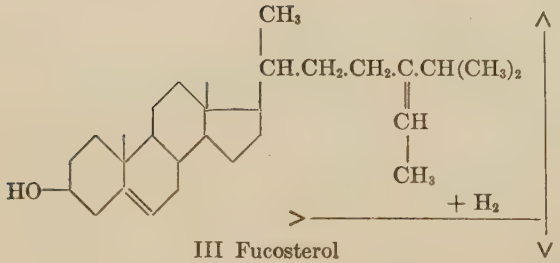
a-series: Chondrillasterol
(Sponges)

b-series: Spinasterol
(Plants)

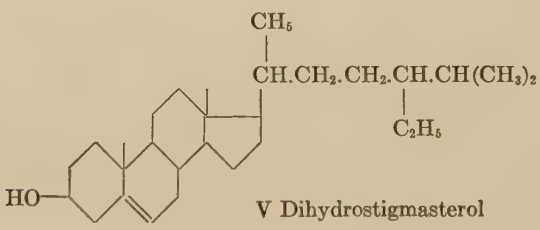
b-series in plants. It is not to be inferred, however, that the presence of one epimer excludes that of another. There exist sufficient indications that in certain plants and marine invertebrates both epimers are present. A possible clue to this interesting dualism of these epimers may be seen in the structure of fucosterol (63), a compound found in many algae (31). This C_{29}-sterol possesses no center of asymmetry at C-24 because of the presence of a double bond at this point. Hydrogenation of this double bond may lead to a C-24-a- or b-ethyl derivative, or both. If one assumes that fucosterol and its C-24-methylene



IV Clionasterol



III Fucosterol



V Dihydrostigmasterol

homolog are intermediates in the biochemical formation of other sterols, one can envisage the hydrogenation of this critical double bond to proceed predominantly in the direction of the a-configuration in marine invertebrates and in the opposite direction in the case of plants.

The sterols represent the major part in the unsaponifiable fractions of most of the marine invertebrates which have been investigated thus far. As mentioned before, this is not always true for the fraction obtained from coelenterates. Here the amounts of higher aliphatic alcohols, such as myristyl- ($C_{14}H_{30}O$), cetyl- ($C_{16}H_{34}O$), and octadecyl alcohol ($C_{18}H_{38}O$), exceed those of the sterols as a rule. In addition, batyl alcohol (16) may be present in significant quantities. This alcohol also forms a substantial part of the unsaponifiable fraction of starfish (19). Finally, in all fractions there are present varying amounts of aliphatic hydrocarbons ranging in consistency from light liquids to solids. More recently the presence of "naphthenic" hydrocarbons and ketones with pronounced optical activity has been indicated. As yet, however, the available data on these constituents of marine invertebrates are too meagre to permit their evaluation on a comparative basis.

PROTOZOA

Dorée envisaged the possibility that sterols are not of primary importance to all forms of life and that animals might be found in whose protoplasm they are absent. As yet no animal devoid of sterols has been discovered; however, it would be premature to interpret such observations as indicating the presence of sterols in all animals. In connection with his significant studies of the lipids of bacteria, Anderson et al. (1) demonstrated convincingly that sterols are absent in tubercle bacilli. In addition Carter et al. (31) have made observations which suggest the same to be true for certain primitive asexual algae, of the class Myxophyceae. On the basis of such information it is reasonable to assume that sterol-free animals, if at all capable of existence, might be found among the most primitive forms, the Protozoa.

Unfortunately, however, our present knowledge of the chemical nature of the lipids of Protozoa is far too limited to permit the drawing of any significant conclusions. The paucity of available information is due primarily to the extraordinary difficulties encountered in securing uniform species of Protozoa in quantities adequate for chemical studies. The two Protozoa about which some reliable data have been made available are listed in Table II. The figures for the

familiar *Noctiluca miliaris* are based on Pratje's (76) studies, for which about 1.5 g. of dry material was available. The author called the sterol, isolated from *Noctiluca*, cholesterol, following the then prevalent custom of assigning this name to any inadequately identified animal sterol. However, until sufficient physical and chemical data are provided for this sterol, its identity will remain uncertain.

TABLE II. Lipid Composition of Protozoa

Class and Species	% of Dry Total fat	% of Fat unsap.	% of Unsapon. sterol	Ref.
Flagellata *Noctiluca miliaris*	12	34.4	24.3	(76)
Sporozoa *Eimeria gadi*	22	36.0	81.5	(74)

More definite data are available for *Eimeria gadi*. Panzer (74) secured a relatively substantial quantity of this coccidium, representing about 80 g. of dry matter, from the air-bladder of the cod, *Gadus virens*. His identification of the sterol as cholesterol is based on sound and adequate evidence.

It must be re-emphasized that the few facts listed above should not be interpreted as indicating the presence of sterols in general, or of cholesterol in particular, in all Protozoa. It is dangerous and frequently misleading to base significant conclusions concerning comparative biochemistry on data derived from the study of but few representatives of a given phylum. This point will become apparent when the great diversity of sterols in Porifera is to be considered in the subsequent chapter.

The cholesterol of the parasitic *Eimeria* may well be of exogenous origin, having been assimilated from the host, in which this sterol is a typical component. Such dependency on host sterols, whatever their nature, may be characteristic for other parasitic protozoa. In nonparasitic forms, however, such as *Noctiluca*, the sterols may well be of endogenous origin and hence of types different from cholesterol. It is to be hoped that more data on the fats of these forms can be accumulated in the future, for without them the general picture of the comparative chemistry of lipids in animals will necessarily remain incomplete.

PORIFERA

In 1904, Henze (46) described the isolation of a sterol from the Mediterranean sponge, *Suberites domuncula*, which was quite different from cholesterol and which he named spongosterol. He therewith demonstrated convincingly the occurrence in animals of sterols other than cholesterol. Dorée, in connection with his systematic studies mentioned previously, discovered a sterol in the cosmopolitan sponge,

Cliona celata (36), which he named clionasterol, having demonstrated not only its difference from cholesterol but also from spongosterol. In 1933 the present author (11) isolated another sterol, microcionasterol, from the abundant New England sponge, *Microciona prolifera*, which again was quite different from the other sponge sterols; a few years later a fourth quite distinct sterol, chalinasterol, was isolated from *Haliclona* (*Chalina*) *oculata* (24). The fact that four different sponges had yielded four different and seemingly new sterols at once indicated a most unusual diversity of sterols in this phylum, and it appeared for a while that every one of the different families, if not genera, might possess a characteristic sterol. Further investigations, however, to be discussed below, showed that such is not the case.

Nevertheless the preliminary observations encouraged the initiation of a systematic study of the sterols and subsequently of the other lipids of sponges. The sponges are particularly well suited for chemical studies, since they may be obtained in adequate and occasionally very substantial quantities and in a sufficient number of different species. Their collection, preservation, storage and extraction in a wet or dry state is readily accomplished. In addition, they furnish sterols in such satisfactory yields as to make possible the elucidation of the structure of new sterols or the separation of complex mixtures.

Since its inception, the purpose of the study of spongesterols has been a dual one. It was anticipated that the study of new sterols would furnish data of interest in connection with the general chemistry of sterols, and in addition it was hoped that some sterol might be found in one of the more abundant sponges, the constitution of which would make possible its ready conversion into hormones or other compounds of pharmacological significance.

The other purpose was to collect as many data as possible concerning the occurrence of sterols in sponges in order to correlate them with the present taxonomy of sponges, which at times appears rather obscure to the uninitiated. The introduction of chemical features into the taxonomy of this phylum does not necessarily constitute a novelty, since the early division of sponges into Keratosa, Silicea and Calcarea has already taken cognizance of chemical differences in sponges.

At present the lipids of more than 50 species of sponges have been investigated. Most of the sponges have been collected personally by the author in the waters of the coasts of New England, of Bermuda, Bimini and Florida.[3]

[3] In this work he has enjoyed the generous assistance of the Bingham Oceanographic Laboratory, the Bermuda Biological Station, the Lerner Marine Laboratory of the American Museum of Natural History, and the Marine Laboratory of the University of Miami. The results of these studies could not be reported had it not

The data concerning the lipids of the sponges which have been investigated thus far are shown in Table III. In accumulating these data it was soon recognized that quantitative determinations are of little significance unless the ash of the sponges is taken into consideration. Most of the sponges contain substantial quantities of foreign inorganic matter such as sand and shell fragments. But for the Keratosa, part of the ash also consists of the sponge spicules. In order to obtain reasonably accurate data relating to the fat content of true sponge material and of its organic fraction, a representative sample of dried ground sponge, weighing 3–10 g., was burnt and the ash determined. Another sample of about equal weight was boiled with concentrated nitric acid until all organic matter had disappeared. In the case of the sponges from Bermuda, Bimini, and to some extent from Florida, such treatment also dissolved the shell fragments and coral sand, leaving only the siliceous spicules. These were then filtered on a glass filter, washed with water, acetone and ether, dried and weighed. The sand present in other sponges may be siliceous and hence remain undissolved in the nitric acid. In order to effect its separation from the spicules, the total undissolved material was agitated with water in which only the spicules are readily, if only temporarily, suspended. Filtration of the suspension and repetition of the process eventually led to the isolation of the spicules in a high state of uniformity.

The spicules of calcareous sponges can not be isolated by this method. Here it is better to digest the sponge material with hot, rather concentrated, sodium hydroxide solution, which dissolves the organic matter. The separation and washing of the spicules is then carried out as described above.

The classification of the sponges listed in Table III is based on that proposed by de Laubenfels in his treatise on the Sponge Fauna of the Dry Tortugas (59). Representatives of the following classes and orders are included in the list.

A. Demospongiae Sollas
- I. Keratosa Bowerbank
- II. Haplosclerina Topsent
- III. Poecilosclerina Topsent
- IV. Halichondrina Vosmaer
- V. Hadromerina Topsent
- VI. Epipolasida Sollas

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been for the enthusiastic co-operation of the eminent sponge taxonomist, Max W. de Laubenfels (Professor of Zoology, University of Hawaii, Honolulu, T. H.), who has identified practically all the sponges under consideration and whose company the author has enjoyed on collecting trips to the Bermudas and Bimini.

TABLE III. The Lipids of Porifera

Species	% of —*Total*— *spic.*	*org.*	*Org. fat*	*Fat unsap.*	*Unsap. sterol*	*Type of sterol*	*Ref.*
AI Spongia obliqua	—	100	14.5	33.7	4.2	10	(27)
AI Oligoceras hemorrhages	—	100	8	35.5	3.1	10	(27)
AI Ircinia variabilis	—	100	4	20	10	10	(27)
AI Verongia fistularis	—	100	4.1	25.5	71	10	(27)
AI Verongia fulva	—	100	6	24.2	44	10	(27)
AI Dysidea crawshayi	—	100	15	33	60	10	(27)
AI Dysidea etheria	—	100	8	——	——	10	(27)
AI Ianthella sp.	—	100	2.5	31	73	10	(27)
AII Spongilla lacustris	—	—	——	——	——	3, 4	(69)
AII Haliclona coerulescens	7	93	2.6	48	67	3, 4	(27)
AII Haliclona variabilis	9	91	5.8	32	63	4	(27)
AII Haliclona rubens	20	80	3.7	76	15	1	(27)
AII Haliclona viridis	26	74	5.8	30	68	1	(27)
AII Haliclona oculata	30	70	5	35	38	7	(24)
AII Haliclona permollis	20	80	14	30	44	3, 4	(27)
AII Haliclona longleyi	44	56	3.5	38	74	9	(27)
AII Desmacidon palmata	74	26	13	32	34	10	(27)
AII Fibulia massa	34	66	3.7	37	——	10	(27)
AII Callyspongia vaginalis	1	99	7.5	19	41	3, 4	(27)
AIII Adocia neens	30	70	5	33	69	10	(27)
AIII Pellina carbonaria	41	59	1.6	——	——	——	(27)
AIII Tedania ignis	24	76	5.7	24	66	3, 4	(27)
AIII Lissodendoryx isodictyalis	14	86	8	36	50	10	(27)
AIII Microciona prolifera	45	55	6	53	48	1, 2	(23)
AIII Zygomycale sp.	55	45	9	33	69	10	(27)
AIV Homaxinella rudis	23	77	4.6	42	——	10	(27)
AIV Pseudaxinella rosacea	16	84	4.4	37	73	10	(27)
AIV Halichondria panicea	—	—	11	37	57	1	(23)
AIV Halichondria melanodocea	40	60	5.4	38	65	1, 2	(27)
AIV Halichondria sp.	65	35	19.3	28	56	2	(27)
AIV Hymeniacidon heliophila	59	41	14.2	38	68	2	(23)
AV Spheciospongia sp.	22	78	4.5	46	50	3, 4	(27)
AV Spheciospongia vesparia	—	—	——	31	70	3, 4	(14,15, 17, 21)
AV Anthosigmella varians	59	41	5.8	48	58	3, 4	(27)
AV Suberites domuncula	—	—	——	——	——	2, 5	(47)
AV Suberites suberea	—	—	——	——	——	2, 5	(27)
AV Suberites compacta	75	25	14	36	70	2, 5	(22)
AV Terpios fugax	21	79	11.2	35	62	2	(27)
AV Terpios zeteki	46	54	8.5	41	64	2	(27)
AV Radiella sol	73	27	11	37	55	2, 10	(27)
AV Polymastia infrapilosa	51	49	11	38	66	2, 10	(27)
AV Weberella bursa	30	70	10.6	64	55	8, 10	(27)
AV Aaptos sp.	17	83	5	33	69	8, 10	(27)
AV Cliona celata	—	—	——	——	——	3, 4	(14, 36)
AV Cliona carriboea	16	84	9.3	27	58	3, 4	(27)
AVI Cryptotethia crypta	20	80	2.1	56	60	10	(27)
AVI Tethya aurantia	33	67	6.3	38	——	10	(27)
AVI Tethya gravida	—	—	10	30	90	10	(27)
AVII Cinachyra cavernosa	43	57	4.5	46	73	10	(27)
AVII Geodia gibberosa	63	37	4.5	32	60	10	(27)
AVII Craniella crania	78	22	7.3	34	80	7	(24)
AVIII Chondrilla nucula	0.5	99.5	3.5	40	86	6	(26)
BI Euplectella sp.	63	37	2.4	——	——	10	(27)
CII Leucetta floridana	46	54	1.3	37	——	10	(27)

VII. Choristida Sollas
VIII. Carnosa Carter
B. Hyalospongiae Vosmaer
I. Hexasterophora Schulze
C. Calcispongiae Schmidt
II. Asconosa de Laubenfels

The individual classes and orders are indicated by numbers in the first column of Table III. The figures in columns 2 and 3 show the considerable variations in spicule content between various species. They are rather typical for a given species and may be reproduced within reasonable limits. Because of such variations, however, it is advisable to compare the fat content of various sponges as calculated on the basis of organic matter rather than the total sponge. Such figures are shown in the fourth column.

So far more than ten different sterols have been isolated from sponges, of which all but two were shown to be new. These sterols are the following: (1) Cholesterol; (2) Cholestanol; (3) Clionasterol; (4) Poriferasterol; (5) Neospongosterol; (6) Chondrillasterol; (7) Chalinasterol; (8) Aaptostanol; (9) Haliclonasterol; (10) X-sterols.

The numbers given in the second to the last column of Table III correspond to the numbers of the sterols in this list. The structures of the first seven sterols have been definitely established and are included in Table I. Sterols 8 and 9 in Table III have been isolated in a high state of purity, but their structures have not been established definitely. In addition, a number of as yet poorly defined sterols have been observed, of which several should eventually turn out to be new. These unknown sterols will be referred to by the number 10 in Table III. The sterol isolated by Mazur (69) from a freshwater sponge, *Spongilla lacustris*, appears to be a mixture of cliona- and poriferasterol (15), and the sponge sterols originally named spongosterol and microcionasterol have been shown to be mixtures (22, 23).

As yet no sponge has been found which is devoid of sterols. However, in three sponges the sterol content of the fat or the unsaponifiable matter has been observed to be substantially below the average. They are *Spongia obliqua*, *Oligoceras hemorrhages* and *Hircinia* (*Ircinia*) *variabilis*, which belong to the order Keratosa.

About ten years ago the author received substantial quantities of commercial and semicommercial sponges, such as "Grass" and "Sheepwool" sponges, from the Bahamas. These sponges had been sliced and air-dried but not prepared as for sale. Extraction of sponges so treated yielded only a very small quantity of fat, which afforded such

a minute amount of sterols as to be hardly noticeable. The impression was gained from these preliminary observations that sponges of the family Spongiidae are very low in fat content. Field studies on Bimini offered the opportunity to repeat the investigations on fresh material. When a freshly caught sponge of the species *Spongia* is injured, it discharges profusely a somewhat viscous liquid, the "gurry." In order to preserve this gurry and to compare its composition with that of the solid material, the following procedure was adopted.

Two freshly caught sponges were cut into small pieces over a dish in which the gurry was collected. The solid was then passed through a meat grinder and the minced material was placed on a large Buchner funnel. By means of heavy weights more gurry was then squeezed out of the solid, a process which took about 24 hours. Toluene was added to the gurry to prevent substantial decomposition. The various fractions of gurry were then combined and dehydrated by codistillation with benzene as described under METHODS. The insoluble residue weighed 93 g., of which 22% was inorganic, mainly sodium chloride. The benzene extract afforded 19.4 g. of fat. The total amount of organic material in the gurry was therefore 92 g., of which the fat represented 21.1%.

The solid sponge material was then treated in a similar manner. It gave 261 g. of ash-free material, of which 12.25% was benzene soluble. The gurry therefore contained 26% of the total organic matter and 40% of the fat of the sponge. When the sponge is dried in the open air in the customary manner the gurry is drained off more or less completely, and unless special precautions are taken it is lost and with it the bulk of the sponge fat. Contrary to the original assumption, therefore, *Spongia* is rich in fat; in fact it is one of the richest so far discovered.

The amount of unsaponifiable matter in the fat is about average, but the sterol content is low, approximately 10% of that of the average sponge fat. The isolation of the sterol has been accomplished so far only by way of the digitonide. A similar condition prevails in *Oligoceras hemorrhages*, another member of the family Spongiidae. Various species of the genus *Hircinia* (*Ircinia*) show similar properties. Here also the gurry contains about one-fourth of the organic matter of the sponge and more than 50% of its fat. The sterol content of the unsaponifiable fraction is also substantially below the average figure. These similarities suggest close biochemical relationships between the three species.

No such complications are encountered in the cases of other *Keratosa*, but although the sterols of several of the more abundant species,

such as *Verongia*, may be obtained in substantial quantities, no definite sterol has yet been identified. As a rule the sterols occur in complex mixtures which are extremely difficult to separate.

Altogether the sterols of more than 50 species of sponges have been isolated, but nevertheless the data are inadequate to permit their correlation to the taxonomy of sponges in general. There appears little doubt, however, that such data will eventually prove their usefulness in supplementing the present taxonomy of the Porifera. Detailed studies on several species of one family of sponges (27) has revealed in them the presence of sterols of such peculiar character as to set this family apart from otherwise taxonomically closely related families. The families to be considered in this connection are the following:

Order: Halichondrina Vosmaer
  Family: Halichondriidae Gray
  Family: Hymeniacidonidae de Laubenfels
Order: Hadromerina Topsent
  Family: Choanitidae de Laubenfels
  Family: Suberitidae Schmidt
  Family: Clionidae Gray

Three species of the Choanitidae (*Spheciospongia vesparia*, *Spheciospongia* sp. and *Anthosigmella varians*) and two of the Clionidae (*Cliona celata* and *C. carriboea*) contain mixtures of clionasterol and poriferasterol. The proportions of the two sterols found in the mixtures of the various sponges are so much alike as to permit no distinction between the mixtures. The sterols or sterol mixtures isolated from nine different species of Suberitidae, however, are quite different. They have one important feature in common, namely the saturated nature of the principal sterol. The presence of a large amount of saturated sterol in a mixture is readily detected. In contrast to the levorotatory unsaturated sterols such as cholesterol, clionasterol, poriferasterol, etc., the saturated sterol shows a distinct positive optical rotation. Thus the observation of the optical rotation of a sterol mixture will at once give some information concerning the amount of saturated sterol present.

The principal saturated sterol of a number of Suberitidae is cholestanol; in others it may be a stanol of the order of C_{28} or C_{29}, the complete identification of which is still lacking. The outstanding fact is that these sterols are saturated regardless of their molecular weight and that they are quite distinct from those found in species from other families of Hadromerina. Such predominance of saturated sterols suggests a sterol metabolism different from that taking place in other families.

The usefulness of sterol analysis in assisting the identification of certain sponges has been demonstrated already in several cases. One of these shall be mentioned as a particularly illustrative example.

A sponge which is rather common in the shallow waters of the southern east coast of the United States, and which is quite abundant in Biscayne Bay, Florida, has been described under a variety of names such as *Suberites distortus*, *S. tuberculosus*, and others. Substantial quantities of this sponge were collected by the author around Miami, Florida, in 1945 and in Bimini in 1948. At first this sponge was identified by de Laubenfels as *S. distortus* on the basis of Schmidt's description (78). But a study of the sterols obtained from a large selection of specimens of this sponge revealed the presence of clionasterol and poriferasterol as well as the absence of any detectable amounts of saturated sterols. From this evidence it was concluded that the sponge in question did not belong to the family Suberitidae but rather to the Choanitidae or Clionidae. In the meantime de Laubenfels had had the opportunity of carrying out field studies on this sponge in Bimini and Florida, which led to the discovery in the sponges of certain microscleres, the presence of which proved the sponge to be of the genus *Anthosigmella* and hence of the family Choanitidae. In many specimens of this sponge the critical microscleres are so few in number that they may easily be overlooked, which may result in misidentification. On the other hand, the sterol analyses give such consistent and reliable results that they must be regarded as most valuable supplementary evidence in the identification of this sponge and of others superficially resembling it.

Another sponge of interest in this connection is *Hymeniacidon heliophila*. This species had been described as *Stylotella heliophila* by Parker (75) and later under the same name by George and Wilson (41). It is quite common near Beaufort, N. C. In 1932, de Laubenfels (58) referred it to *Hymeniacidon* because of its close resemblance to various European members of this genus. If this sponge were indeed a species of the genus *Stylotella* it would belong to the family Suberitidae of the Order Hadromerina, and hence one should expect it to contain a saturated sterol as the principal component of its sterol mixture. Otherwise it would belong to the family Hymeniacidonidae of the order Halichondrina.

Analysis of the sterol mixture that was obtained from a large amount of this sponge revealed the presence of a saturated sterol, cholestanol (23), as the principal sterol. On the basis of this evidence, therefore, it might be argued that the sponge under consideration is a species of Suberitidae and that it should be referred once more to this family.

Such a transfer will remain premature, however, until more is known about the sterol content of other species of *Hymeniacidon* and of the closely related *Halichondria*. Until such knowledge is available it is safe to state only that there appear to exist closer chemical relationships between *Hymeniacidon heliophila* and the Suberitidae than between the latter and Choanitidae and Clionidae.

At present the sterols of seven species of the genus *Haliclona* (Order Haplosclerina) have been isolated and investigated. The results of these studies have revealed a surprising lack of uniformity of the sterols or sterol mixtures present in these species. Each of three species contains a different sterol in an astonishingly high degree of uniformity. Thus *Haliclona oculata* has afforded chalinasterol (24), which is identical with the ostreasterol found in certain mollusks (5). Poriferasterol has been shown to be the principal sterol of *Haliclona variabilis* (27). A new sterol, which has tentatively been named haliclonasterol, $C_{28}H_{48}O$, is present to the practical exclusion of other sterols in *Haliclona longleyi* (27). The sterol mixtures isolated from *Haliclona viridis* and *H. rubens* are of a similar composition, their most outstanding feature being the presence of more than 50% of cholesterol. Both *Haliclona permollis* and *H. coerulescens* have yielded sterol mixtures in which poriferasterol and clionasterol appear to be the principal components.

At present the significance of these observations is difficult to evaluate. The necessity of a revision of this genus, into which approximately 100 species have been placed, is generally realized. The probability is envisaged here that in such a future revision sterol analyses will render valuable assistance. On the basis of the present, but unfortunately limited, evidence derived from sterol studies, there appear to exist at least four distinct groups within the genus *Haliclona*. They are I. the *oculata* type, II. the *longleyi* type, III. the *rubens* and *viridis* type, and IV. the *permollis* and *coerulescens* type. The last group might possibly include *Haliclona variabilis* also, if the difference between its sterol mixture and that of the two others is regarded as of a quantitative rather than a qualitative nature.

COELENTERATA

Table IV lists the coelenterates which at present have been subjected to more than a casual study and lipids obtained from them. All but two of the species belong to the Anthozoa which, for technical reasons, are best suited for chemical studies. The fat content of the coelenterates is rather high, particularly when calculated on the basis of the organic matter of the animal rather than its total weight. With the

exception of *Velella spirans*, the unsaponifiable fraction of coelenterate fats is almost as high as that of sponge fats. It has been pointed out in the previous chapter that the sterols form the most substantial part of the unsaponifiable fraction of sponge fats, but this is not the case in the corresponding fraction from coelenterates. Here the sterols are frequently present in such comparatively small amounts as to make

TABLE IV. LIPIDS OF COELENTERATA

Class and Species	% of *Total* org.	% of *Total* fat	% of *Org. fat*	% of *Fat unsap.*	% of *Unsap. sterol*	*Ref.*
Hydrozoa						
Millepora alcicornis	——	0.25	——	43	10	(27)
Velella spirans	53	15	27	5.3	—	(45)
Anthozoa						
Gorgonia flabellum	——	2.3	——	39	35	(27)
Plexaura flexuosa	14	1.7	12.2	36	—	(18)
Plexaura dichotoma	10	2.5	23.5	34	—	(27)
Pseudoplexaura crassa	——	2.1	17.5	39	—	(27)
Eunicea Tourneforti	12	2.3	19.4	46	—	(27)
Condylactis gigantea	——	——	33	24	4	(27)
Urticina nodosa	——	——	7	32	—	(27)
Zoanthus proteus	——	——	7	39	—	(27)
Palythoa mammilosa	——	——	2.3	33	41	(27)
Protopalythoa grandis	——	——	3.2	37	35	(27)
Meandra areolata	——	——	——	——	—	(18)
Meandra cerebriformis	1.5	0.15	10	41	6	(27)
Mussa fragilis	——	0.5	——	41	5	(27)
Oculina diffusa	——	0.3	——	24	9	(27)
Siderastrea radians	——	0.3	——	26	11	(27)
Madrepora cervicornis	——	0.5	——	33	—	(18)

difficult their isolation by means other than precipitation with digitonin. The bulk of the unsaponifiable matter consists of higher alcohols and hydrocarbons of the types which have been listed under METHODS. The fats of a number of coelenterates are rich in "wax-like" matter, such as cetyl palmitate (13), the saponification of which accounts for the presence of cetyl alcohol in the unsaponifiable fraction. The occurrence of other waxes, such as fatty acid esters of myristyl- and octadecylalcohol, is indicated by the presence of such alcohols in sea anemones (27) and gorgonids (16).

As yet little is known about the nature of the sterols of coelenterates. The only sterols which have been definitely identified so far are cholesterol and chalinasterol. Cholesterol has been found in certain sea anemones (36), in a coral, a gorgonid (18) and a jellyfish (45), chalinasterol in the colonial anemone, *Zoanthus proteus* (27). A new sterol, tentatively named palysterol, has been isolated from another colonial anemone, *Palythoa mammilosa* (27). Its structural relations to other sterols have not yet been established. The actiniasterol (55) and gorgoniasterol (18) which were regarded originally as new sterols have

since been shown to be complex mixtures of ill-identified sterols (27, 35). The Provitamin-D content of the sterols of a number of coelenterates has been determined spectrographically (28, 42); it ranges from 2–10%. These data do not include any statements concerning the general nature of the sterols, and therefore they are of interest only in connection with the Vitamin-D problem.

Except for the lipids of the few species listed in Table IV, practically nothing is known about the organic constituents of stony corals. Contrary to popular belief, reef-building corals contain by no means inconsiderable quantities of organic matter. This was first pointed out in 1846 by Silliman (80), who reported in his classical paper on the composition of stony corals; he found that the organic matter represented from 4–8% of the total and that it was intimately united with the inorganic material throughout the structure of the coral. His observations were verified in 1922 by Clarke and Wheeler (32), who determined the organic matter of 28 species of corals and found that it amounted to from 2–7% of the total. Similar observations have been made quite recently by the present author (27).

Silliman was also the first to call attention to the fact that part of the organic matter of corals consists of some wax-like material which can be separated from its surroundings by boiling water. After coral fragments had been boiled, he found this wax floating on the surface of the water in transparent, jelly-like masses of yellowish color. It was insoluble in alcohol but readily soluble in cold ether, and the evaporation of its ethereal solution yielded a solid which resembled "wax." The average amount of such wax-like fats in stony corals is about 0.3% for species from Bermuda and probably 0.5% for those from more tropical regions. As Silliman had already pointed out, this lipid is not confined to the thin living layer of the coral but extends deep into the entire structure. The weight of the total fatty material of a massive coral of one cubic meter volume, such as a brain coral, may be calculated to be in excess of seven kilograms, and that of a coral reef to be of substantial magnitude.

Such considerations led the present author and Lester in 1940 (12) to call attention to coral reefs as one of the possible sources of petroleum. They maintained that, unless very unusual conditions prevail, the bulk of organic matter from dead marine animals and plants is probably brought back rapidly into circulation in one way or another. In the case of reef-building organisms, including bryozoa and coralline algae, a significant portion of the organic matter becomes trapped in the evergrowing inorganic mass and hence is removed from circulation. If this is the case, then reefs [bioherms (34)] must be regarded as vast

accumulators of materials which may be considered as potential precursors of petroleum. At some time comparatively mild changes in the physical conditions may loosen this material from its inorganic surroundings and bring it to the surface, as in the case of the wax-like material which Silliman found floating on the surface of the water in which corals had been boiled.

In this connection it is of interest to note that in February 1947 a commercial oil pool was discovered near the village of Leduc, Alberta. This pool has been described recently by Link (60), who holds that "the oil and gas accumulation is apparently due primarily to a stratigraphic trap created by coral-reef or bioherm development." He also points out that at present the majority of petroleum geologists would probably consider the surrounding shale and the underlying sediments as the source rock of the oil accumulated at Leduc. In his opinion, however, "the oil was not only stored in the bioherm but was also generated in the reef."

PLATYHELMINTHES AND NEMATHELMINTHES

With but few exceptions, nothing appears to be known about the lipids of animals belonging to phyla generally placed between the Coelenterata and Annelida. Substantial quantities of cholesterol have been isolated from the parasitic flatworms, *Diphyllobothrium latum* (*Bothriocephalus latus*) by Faust and Tallquist (38) and Welsch (95), *Fasciola hepatica* by Brand (30) and *Taenia taeniaformis* by Salisbury and Anderson (77). The nematodes, *Ascaris lumbricoides* and *A. megalocephala*, have been studied by Flury (40), who found that the dried worms contained 10.9% of fat, of which 24.7% was unsaponifiable.

ANNELIDA

For centuries an oil extracted from the earthworm *Perichaeta communissima* has been used in Japan as an antipyretic. Because of its alleged medicinal value the oil was investigated by Murayama and Aoyama (72), who found that it represents 2.3% of the dry animal and that it contains 31.2% of unsaponifiable matter. A similarly high amount of this fraction has been found in other earthworms and also in two marine species, as shown in Table V.

As yet only the sterols of *Lumbricus terrestris* have been adequately investigated. De Waele (94) has shown that the principal sterol is cholesterol, but Bock and Wetter (28) have demonstrated more recently that the original sterol mixture contains, in addition to cholesterol, more than 20% of a Provitamin-D, identified as ergosterol, and also some other poorly defined sterol or sterols.

TABLE V. Lipids of Annelida

Class and Species	*% of Fat Unsaponifiable*	*Ref.*
Oligochaeta		
Perichaeta communissima	31.2	(72)
Lumbricus terrestris	36.7	(94)
Lumbricus rubellus	21.3	(71)
Tubifex tubifex	10.5	(71)
Polychaeta		
Aphrodite hastata	23.6	(27)
Nereis pelagica	16.2	(27)
Hirudinea		
Hirudo medicinalis	18.2	(71)

CRUSTACEA

Despite the fact that crustaceans are readily available in a profusion of species, practically no systematic studies have been carried out on the composition of their fatty constituents. There even exists a paucity of information concerning the amount of unsaponifiable matter present in the fats. The data shown in Table VI have been

TABLE VI. Lipids of Crustacea

Species	*% of Fat Unsaponifiable*	*Ref.*
Daphnia galeata	22.3	(62)
Daphnia magna	13.5	(71)
Leptodera Kindtii	16.7	(71)
Carcinogammarus sp.	11.5	(71)
Copepod plankton	26.3	(33)
Calanus finmarchicus	32	(62)
Cyclops stremus	20	(62)
Diaptomus gracilis	17.5	(62)
Ligia exotica	11.3	(82)
Potamobius fluviatilis	12.2	(71)
Cambarus affinis	14	(71)
Eriocheir sinensis	5.9	(71)
Crangon crangon	13.6	(71)
Leander adspersus	15.1	(71)

derived largely from planktonic forms, many of these being species that inhabit fresh water. Corresponding data for the more conspicuous marine forms, such as crabs, lobsters, etc. are still missing.

All available evidence indicates that cholesterol is the typical sterol of crustaceans. Dorée (36) first showed it to be the constituent of a crab, *Carcinus moenas*. Later cholesterol was isolated from *Portunus plicatus* and *Eriphia spinifrons* by Leulier and Policard (61), and quite recently from *Cancer magister* by Kind and Fasolino (52). The presence of substantial amounts of cholesterol in the oil of a *Cypridina* species has been shown by Kotake and Kimoto (57); Lovern (62) has

indicated its presence in the unsaponifiable matter of copepod oil, where it is accompanied by a large amount of alcohols, such as cetyl alcohol and higher homologs. Cholesterol has been isolated also from *Ligia exotica* (82). Of interest in this connection is the work by Vilbrandt and Abernethy (93) on the utilization of shrimp waste. These authors found that such waste contains 2.25% of an oil from which 19% of cholesterol can be extracted without taking recourse to saponification. They have pointed out that "a production of 80,000 pounds of cholesterol could have been accomplished by the extraction of the waste of the 1927 crop of shrimps."

MYRIAPODA

The fatty extract of the millipede, *Strongylosoma tambanum*, which has found some use in Japan under the name of "Aka-yusade" oil, contains 21.4% of unsaponifiable matter. Ueno and Yamasaki (89) have isolated a sterol from this fraction which they named yasudesterol in the belief that it was a new compound. However, the evidence presented by the authors is indicative of lack of uniformity in the sterol. Aside from this isolated fact, nothing appears to be known about the lipids of this class of animals.

INSECTA

The fats of insects have been the subject of numerous investigations, but systematic studies on the unsaponifiable fractions and the sterols they contain are still missing. Data have become available on the amounts of unsaponifiable matter in the fats of more than 30 species. They have been contributed principally by Mieller (71), Timon-David (81), Giral (43), Welsch (95), Ueno and Komori (90), Tsujimoto (84) and Bergmann (6). The lowest value is reported for *Ergates faber*, 0.75% (80), the highest for *Melolontha vulgaris*, 19.4% (95). The average of 31 values is about 7%.

The only insect fat of commercial value is the "chrysalis oil," a by-product of sericulture. Because it is readily available, it has served as the first starting material for the study of insect sterols. It was originally believed that the principal sterol of this oil from *Bombyx mori* was a new sterol, and consequently it had been named bombicysterol. More recent investigations by Bergmann (6) have shown, however, that this sterol is a mixture in which cholesterol is the principal component. The cholesterol is accompanied by other sterols, the nature of which has not yet been established. It appears quite certain that Kawasaki's inagosterol from locusts (51) is a mixture of similar composition. All available evidence indicates that analogous

mixtures are present in other herbivorous insects, and that cholesterol may be regarded as the typical sterol for all insects.

XIPHOSURA

The sterol of the horseshoe crab, *Limulus polyphemus*, is cholesterol, as has been shown by Bergmann et al. (18). Nothing appears to be known about the lipids of Arachnida.

MOLLUSCA

In 1934, Bergmann (4, 5) and Tsujimoto and Koyanagi (87) independently presented convincing evidence that the principal sterols of certain mollusks may be other than cholesterol. Since then a considerable amount of information on mollusk sterols has become available, the pertinent data of which have been summarized in Table VII. These data show a surprising constancy of the fat content of

TABLE VII. LIPIDS OF MOLLUSCA

Class and Species	*% Fat of wet weight*	*% of Unsap.*	*Sterylacetate M. P. °C. and sterol*	*Ref.*
Pelecypoda				
Tapes phillippinarum	0.8	15.3	137	(85)
Corbicula leana	0.7	14.8	126–7 corbi-, brassicasterol	(64, 67, 85)
Cristaria plicata	1.3	13	137–8	(85)
Meretrix meretrix	0.7	12.6	137–8 meretristerol	(83)
Ostrea gigas	1.4	11.8	136–7 conchasterol	(68, 87)
Ostrea virginica	1.5	11	134–5 ostreasterol	(4)
Mya arenaria	1.6	——	131	(87)
Volsella modiolus	3 (?)	12	127–8	(87)
Tridacna gigas	0.9	11.5	156–7 shakosterol	(86)
Venus mercenaria	0.7	15	131	(4)
Modiolus modiolus	1.0	16	131	(4)
Modiolus demissus	——	——	156–7 brassicasterol	(27)
Gastropoda				
Haliotis gigantea	1.0	10	117 cholesterol	(85)
Turbo cornutus	1.0	14.6	116 cholesterol	(85)
Rapana thomasiana	1.8	15	120 cholesterol	(87)
Cellana nigrolineata	4.2	15	115 cholesterol	(87)
Tegula xanthostigma	2.8	11	118 cholesterol	(86)
Fulgur sp.	1.5	12.3	117 cholesterol	(4)
Buccinum undatum	——	——	—— cholesterol	(28, 36)
Littorina littorea	——	——	—— cholesterol	(53)
Nerita peleronta	——	——	—— cholesterol	(54)
Nassa obsoleta	——	——	—— chloesterol	(54)
Cephalopoda				
Sepia officinalis	——	——	—— cholesterol	(35, 48)
Octopus vulgaris	——	——	—— cholesterol	(95)

various fresh wet mollusks and of the amount of unsaponifiable matter present in the fats. The average fat content is somewhat higher in gastropods than in pelecypods. In disagreement with these figures are those shown in Table VIII which have been taken from Mieller's

(71) little known report on the fats of invertebrates. The average of Mieller's figures for percentages of unsaponifiable matter is approximately twice that of those shown in Table VII. This discrepancy, which is well outside the limits of experimental error, may find an explanation in the fact that all species investigated by Mieller are inhabitants of fresh water, while those listed in Table VII are of marine origin with the exception of two terrestrial snails.

TABLE VIII. Lipids of Freshwater Mollusks (71)

Species	*% of Fat Unsaponifiable*
Limnaea stagnalis	29.8
Planorbis corneus	24.1
Paludina vivipara	35.1
Unio sp.	20.8
Anodonta sp.	24.1
Dreissensia polymorpha	14.0
Sphaerium sp.	24.2

The first mollusk sterol to be recognized as different from cholesterol was ostreasterol (4). At first it was believed to be a typical constituent of all mollusks. Later investigations, however, revealed in a variety of species the presence of other sterols, among them cholesterol. The confusion resulting from such seemingly contradictory observations is cleared up in part when the species are arranged according to classes. It then becomes apparent that in the pelecypods the sterols other than cholesterol predominate or are present in substantial amounts. In contrast, the gastropods contain cholesterol as their principal sterol. It must be emphasized that these differences are of a quantitative rather than qualitative nature, since the presence of cholesterol has been detected in pelecypods (37), and that of sterols other than cholesterol in gastropods. Nevertheless, the over-all difference is quite distinct and merits special attention. Unfortunately nothing appears to be known as yet about the sterols of freshwater mollusks.

In most cases the melting point of the mollusk sterylacetate already affords a good indication of the nature of the principal sterol. A melting point between 110–120° C points to the presence of cholesterylacetate, m. p. 115–116° C, the melting points above 130° to that of other mollusk steryl acetates (see Table VII). These new mollusk sterols have been described under the following names: ostreasterol, shakosterol, conchasterol, meretristerol, magakisterol and corbisterol. The structure of two of these sterols has been definitely established. Ostreasterol has been shown to be identical with chalinasterol (Table

I) (25), which in turn has been identified as the C-24-methyl epimer of brassicasterol. The properties of shakosterol (88) are so similar to those of brassicasterol as to leave little doubt about the identity of the two compounds. Moreover, the occurrence of brassicasterol in mollusks has been demonstrated convincingly, and it has been shown also that this sterol had once been mistaken for stigmasterol (8, 27). The suggestion has been made that meretristerol and conchasterol (67) are identical, but it appears doubtful that they are uniform compounds. The probability is strongly indicated that they are mixtures, difficult to separate, of the two epimers chalina- and brassicasterol. Too little is known about magakisterol (67) to permit any prediction concerning its constitution, but it is probably a mixture of substances. Corbisterol (64) appears to be a sterol of the Provitamin-D type, as has been suggested recently by the present author (27). The presence in mollusks of substantial amounts of sterols acquiring antirachitic activity upon irradiation has been known for some time. Thus Bock and Wetter (28) and Boer et al. (29) have shown that the Provitamin-D content (ergosterol) of the sterol mixture was 9.3–25% in the land snails *Helix pomatia* and *Arion empiricorum* and 5–26% in marine mollusks, *Littorina littorea*, *Archidoris tuberculata* and *Mytilus edulis*. The Provitamin-D content of the abundant mussels *Mytilus* and *Modiolus* has been found sufficiently high to make commercial use of these species in Vitamin-D preparations. The nature of the Provitamin-D from *Mytilus* has been thoroughly investigated recently by van der Vliet (91, 92), who believes the principal components to be ergosterol and 7-dehydrocholesterol.

ECHINODERMATA

At present reliable information on the lipids of echinoderms has become available for three of the five classes of this phylum. The pertinent data are given in Table IX. The figures given for the fat content of the various species are as yet of little comparative value. They are based on the total animal rather than its organic matter. The values for the amounts of unsaponifiable material present in the fat are more significant; their average is about 20%.

The sterols of Asteroidea were among the first animal sterols to be recognized as distinctly different from cholesterol. Dorée (36) called attention to their peculiar properties, and later Kossel and Edlbacher (56) described one of them in greater detail, naming it stellasterol. More recent investigators have assigned other names to these sterols, such as asteriasterol (73) and hitodesterol (66). The structure of these sterols was eventually elucidated by Bergmann (7, 20), who showed that the typical mixture contains a mono-unsaturated sterol,

stellastenol, and a di-unsaturated setrol, stellasterol. Both sterols are C-24-a-methyl sterols of the type shown in Table I. These sterols are easily differentiated from cholesterol by the high melting point of their acetate, by their optical rotations, which lie close to zero, and by the green color reaction which they give with bromine. Strong indications exist that the same sterol mixture, or one closely related to it, is also present in the Holothuroidea (18).

TABLE IX. LIPIDS OF ECHINODERMATA

Class and Species	*% of Fat Unsaponofiable*	*Type of Principal Sterol*	*Ref.*
Asteroidea			
Asterias rubens	39	stellasterols	(56, 49)
Asterias forbesi	34	stellasterols	(7, 20)
Asterias rollestoni	10	hitodesterol	(66)
Astropecten scoparius	12	hitodesterol	(82)
Asterina pectinifera	20	hitodesterol	(82, 65)
Echinoidea			
Tripneustes esculentus	26	cholesterol	(18)
Centrichinus antillarum	20	cholesterol	(18)
Lytechinus variegatus	—	cholesterol	(18)
Heliocidarus crassidus	—	cholesterol	(82)
Arbacia punctulata	22	cholesterol	(27)
Holothuroidea			
Holothuria princepo	11	stellasterols	(18)
Cucumaria chronjhelmi	11	stellasterols (?)	(66)

In contrast, the principal sterol of all Echinoidea which have been studied so far is cholesterol (18, 52). Its presence has been demonstrated convincingly in four species and has been indicated in others.

The striking difference in the nature of the sterols of Asteroidea and Holothuroidea on one side and that of Echinoidea on the other is to some extent paralleled by another constituent of the unsaponifiable fraction. As has been mentioned under METHODS, the glycerol ether, batyl alcohol, is a typical constituent of the unsaponifiable fraction of starfish fat. This compound (56, 19) may occur in such quantities as to obscure the presence of the sterol.

Recently Matsumota and Toyama (66) have shown that batyl alcohol, or a closely related compound, is also present in substantial amounts in the unsaponifiable fraction of the holothurian, *Cucumaria chronjhelmi*. In the corresponding fractions of Echinoidea the presence of such compounds has not yet been observed. If they are present at all, and it seems likely that they might be, it will be in quantities significantly smaller than those found in representatives of the other two classes.

In his discussion of the distribution of arginine and creatine in animals, Baldwin (2) has called attention to the fact that of all the invertebrate types studied prior to 1947 "the echinoderms are excep-

tional in that they alone include species in which the presence of creatine has been satisfactorily demonstrated."[4] In the echinoids, arginine and creatine occur side by side, while in the ophiuroids (brittle stars) only creatine could be detected.

TABLE X. Distribution of Phosphagens, Sterols and Batylalcohol in Echinodermata

	Arginine	*Creatine*	*Stellasterol*	*Cholesterol*	*Batylalcohol*
Asteroidea	+	−	+	−	abundant
Ophiuroidea	−	+	−	+ (?)	?
Echinoidea	+	+	−	+	absent (?)
Holothuroidea	+	−	+	−	abundant

In Table X the data given by Baldwin (2) are compared with those based on the study of the sterols. The fact stands out that, on the basis of three quite different and independent sets of chemical analyses, the asteroids and holothurians may be regarded as a group set apart from the echinoids. It is of considerable interest that cholesterol is the principal sterol of the echinoids. The same compound is the typical sterol of the vertebrates, and on the basis of limited information it also appears to be characteristic for Protochordata. Since it has been shown that certain anatomical relationships exist between echinoids and Protochordata, the presence of cholesterol in the former appears to be of particular interest.

Unfortunately no data are available on the sterols of crinoids for a completion of the picture. Only one observation has been made so far on the sterols of the ophiuroids, *Ophiopholis aculeata* (27), and this will have to be accepted with some reservation until it has been verified. The total amount of available sterol was only about 40 mg. It was distinctly different from the starfish sterol, as shown particularly by its strongly negative rotation of −36°. The identity of this sterol with cholesterol has been indicated but not fully demonstrated because of lack of material. If further studies should prove the sterol of ophiuroids to be cholesterol, then another striking parallel between the occurrence of arginine-creatine and stellasterols-cholesterol will have been established for this phylum. It would indicate also that a closer biochemical relationship exists between the ophiuroids and echinoids than between the former and the asteroids.

In this connection a recent paper[5] by Fell (38a), which deals with echinoderm embryology, is of particular interest. In it the author has

[4] See, however, a recent paper by Baldwin and Yudkin (3), in which the presence of creatine in annelids is indicated.

[5] The author is indebted to G. E. Hutchinson for having called his attention to this paper.

presented "a phylogenetic tree [Fig. 3] which the recapitulation theory would construct from embryological evidence, if larval forms are regarded as repeating ancestral conditions."

However, Fell arrives at the conclusion that "it is impossible to accept the result which implies that ophiuroids and echinoids are more closely related to each other than to other classes, and that the

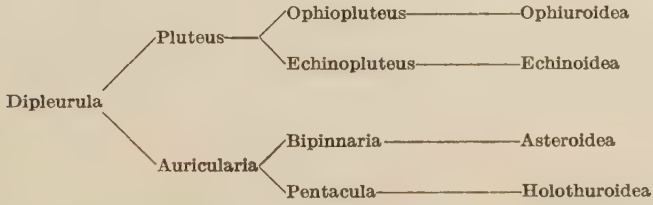

FIGURE 3. Phylogenetic tree of Echinoderms based on Embryological evidence.

holothurians and starfish are similarly connected," and that "the recapitulation theory as applied to larval forms leads to a *reductio ad absurdum* in the case of echinoderms." Looked at from the viewpoint of comparative biochemistry, this phylogenetic tree does not appear as absurd as indicated by Fell. On the contrary, the embryological evidence which favors close relationship between holothurians and starfish on the one side, and between echinoids and ophiuroids on the other, finds substantial support in biochemical evidence which indicates analogous relationships.

TUNICATA

Except for the observation by Bergmann et al. (18) that cholesterol is the principal sterol of the tunicate, *Styela plicata*, nothing appears to be known about the unsaponifiable matter of Protochordata.

VERTEBRATA

In contrast to the fats of invertebrates, those of vertebrates contain only small quantities of unsaponifiable matter. The average amount of this fraction in the fats of more than 50 species ranging from the lamprey to man is about 1.2%. In calculating this average value, the oils of a few species have not been included because they are known to contain exceptionally large amounts of unsaponifiable matter. These are, for example, the liver oils of sharks which may contain in excess of 80% of hydrocarbons and the head oils of the sperm whale and related species which consist principally of cetyl palmitate.

The sterols of vertebrates have been the subject of numerous investigations. In all species, cholesterol has been found to be the principal sterol, accompanied by only small amounts of cholestanol and 7-dehydrocholesterol. Hüttel and Behringer (50), however, have found by no means inconsiderable quantities of phytosterol-like compounds in fats extracted from the skins of certain toads. Thus the sterol mixtures from the skins of *Bufo vulgaris*, *B. vulgaris formosa* and *B. arenarium* contain 20, 5 and 17% of such sterols respectively. This observation emphasizes the peculiar character of the biochemistry of the toad in which the synthesis of "digitalis-like" heart poisons and the excretion of arginine are prominent features.

DISCUSSION

The most outstanding difference between the fats of vertebrates and invertebrates is the presence in the latter of substantial amounts of unsaponifiable material (see Table XI). In 1923, at a time when only a few data were known, Grün (44) had already called attention to this

TABLE XI. Distribution of Unsaponifiable Matter in Animals

Phyla or Classes	*% of Unsaponifiable Matter of Fat*	*Number of Examples*
Protozoa	35	2
Porifera	37	45
Coelenterata	35	16
Nemathelminthes	25	1
Annelida	22	7
Crustacea	16	14
Myriapoda	21	1
Insecta	7	31
Mollusca (marine)	13	18
Echinodermata	19	10
Chordata	1.2	50

fact, and in addition he made certain predictions and drew certain conclusions which are of general interest. His comments appear to have been widely overlooked, principally because they form but a small fraction of a long review on the progress of fat chemistry, which has been published in a trade journal read only by a limited group of scientists. In order to make his comments better known, a complete translation of the pertinent paragraph is given herewith.

A systematic study of the fats of lower animals and plants might lead to the discovery of relationships which should prove to be of biological interest, in particular as far as the theory of evolution is concerned. In the lowest animals and plants, also in bacteria, one finds in contrast to the higher forms less glycerides than wax-alcohols and hydrocarbons. (There are, however, also vertebrates, whose liver fats consist up to 90% of hydrocarbons. They are interestingly enough

the Elasmobranchs which are most closely related to the Proselachii.) It is also to be taken into consideration that the fats of oil producing plants of higher order in their earlier processes of development consist mainly of wax-like compounds which make room for the glycerides as the plant matures until they have practically disappeared. (See for example the maturation of the "poppy-milk," hemp etc.) Since the biogenetic law teaches that the development of the individual is a rapid recapitulation of the development of the species, the composition of the fat of a youthful plant of higher orders may serve as an index to the composition of the fat of primitive precursors or the "Urform" in general.

The data which have been accumulated since the appearance of Grün's paper support his views in the difference between the fats of lower and higher animals. In addition, they indicate that among the invertebrates the lower forms have the higher amounts of unsaponifiable matter in their fats. It appears, therefore, that evolution has been accompanied by a decrease in the relative amounts of this fraction. It is to be hoped that future systematic studies will contribute more data needed to complete this fragmentary picture.

The relatively low figure for the amount of unsaponifiable matter in insect fats should not be interpreted as typical for terrestrial invertebrates in general. The fats of other terrestrial invertebrates may contain such material at the usual high level, such as 21.5% in the case of a millepede and 30% in that of earthworms.

TABLE XII. Distribution of Sterols in Animals

Phyla and Classes	*Principal Sterols*
Porifera	cholesterol, cholestanol, clionasterol, poriferasterol, chalinasterol, neospongosterol, chondrillasterol, haliclonasterol, aptostanol and others.
Coelenterata	cholesterol, clionasterol, chalinasterol, palysterol and others.
Annelida	cholesterol
Arthropoda	cholesterol
Mollusca	
Pelecypoda	chalinasterol, brassicasterol, corbisterol, cholesterol and others.
Gastropoda	cholesterol
Cephalopoda	cholesterol
Echinodermata	
Asteroidea	stellasterols
Holothuroidea	stellasterols
Echinoidea	cholesterol
Protochordata	cholesterol
Chordata	cholesterol

Table XII presents a summary of the results of comparative studies on the sterols of animals. It is the summary of a story which is still far from completion. As yet it does not include species of several phyla, while species from other phyla are represented in numbers too inadequate to permit the drawing of far reaching conclusions. It is to be expected, therefore, that the general picture, as it now appears, will

undergo many modifications as soon as more data become available. Nevertheless it can be stated with some certainty already that the greatest diversity of sterols is to be found among the more primitive invertebrates, and that the process of the biochemical evolution of the sterols has led into the direction of the practically exclusive use of cholesterol.

For some time it had been customary among sterol chemists to divide sterols on the basis of their natural occurrence into animal and plant sterols, zoo- and phytosterols. It was then believed that animals were incapable of synthesizing the complex ring system of the sterols, and that they assimilated such compounds from plant material in order to modify them into cholesterol. Schoenheimer (79) and others, however, showed eventually that such is not the case, and that cholesterol is of endogenous origin. In the meantime it had been discovered also that plant sterols are not of the same order C_{27} as cholesterol, as formerly believed, but of the higher orders C_{28} and C_{29}. This discovery appeared to re-emphasize the difference between plant and animal sterols.

In the beginning of his studies on the sterols of invertebrates, the present author observed the presence of a phytosterol-like sterol in the oyster, *Ostrea virginica*, and of cholesterol in the marine gastropod, *Fulgar* sp. He then interpreted the differences in sterol content of the two species as due to the exogenous origin of sterols in mollusks. The herbivorous oyster was thought to assimilate the plant sterols of its phytoplankton diet, and the carnivorous snail to utilize the cholesterol of its diet. Such assumptions eventually led to the formulation of the tentative hypothesis that invertebrates, and in particular marine invertebrates, are incapable of synthesizing sterols, and that the sterols found in them are of exogenous origin and hence related to the sterol of the diet. Subsequent observations seemed to support this hypothesis for a while, such as the presence of cholesterol in certain carnivorous coelenterates and the presence of phytosterol-like compounds in the herbivorous sponges. However, as more data became available this hypothesis gradually became untenable. The discovery of substantial amounts of cholesterol in the herbivorous silkworm and the earthworm had already made necessary a restriction of this hypothesis to marine invertebrates. It had to be abandoned when sterols of the order C_{28} were found in the carnivorous starfish (20) and cholesterol in sponges and in such herbivorous gastropods as *Haliotis* (86).

Therefore, it appears at present that, with the possible exception of parasitic species, all animals are capable of producing their own sterols. As mentioned before, the lowest forms synthesize the greatest

variety of sterols, including cholesterol, the highest only cholesterol and some of its derivative in minor amounts. Such a reduction of a multitude of closely related compounds which seemingly perform closely related functions to a few compounds, if not only one, appears to be a general phenomenon of biochemical evolution. If this were the case one should expect *a priori* that the distribution of the phosphagens, creatine and arginine phosphoric acid is not as clearly divided among vertebrates and invertebrates as had been believed originally. On the contrary, one should expect the presence of both phosphagens in species of the lower phyla, with the presence of additional phosphagens, such as agmatine phosphoric acid or as yet unknown types, in species of the lowest phyla. Indeed the recent observations by Baldwin and Yudkin (3) on the phosphagens of annelids appear to support this view. In general, therefore, evolutionary development seems to be accompanied, if not caused, by a reduction in the number of chemical products and reactions and by retaining only those which have proved to be the most suitable, the most efficient.

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SAMPLING ATMOSPHERIC SEA-SALT NUCLEI OVER THE OCEAN[1]

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Atmospheric hygroscopic nuclei are of primary interest to meteorologists in studies of haze, fog, clouds, etc. (Simpson, 1941; Middleton, 1941; Sheppard, 1947). The sea surface is presumed to be an important source of these nuclei in the form of drops of concentrated sea water arising from spray. Gautier (1899), Jacobs (1939) and others have made measurements of total air-borne chlorides by use of filtering methods; Wright (1940b) and Neiburger and Wurtele (1948) have related changes in visibility and relative humidity to the number and average size of hygroscopic nuclei. However, no information seems to be available which gives the measured weights and numbers of sea-salt nuclei[2] at various heights in clear air over the open sea (see summaries by Landsberg, 1938; Wright, 1940a; Simpson, 1941).

The primary purpose of this paper is to describe the methods used to sample sea-salt nuclei in air over the sea and to measure the weight of chloride[3] in these nuclei. Data are shown giving the weight and number of salt nuclei sampled from aircraft at various heights over the open sea.

The following methods of sampling and measuring air-borne nuclei containing chlorides are modifications of those used by Cunningham (1942). The sampling of aerosols by exposing small glass slides to an air stream has been used by Houghton and Radford (1938), Cunningham (1942), May (1945), Schaefer (1946) and many others. The present authors used 1 x 15 mm glass surfaces cut from microscope slides that were ground to size on a lens-grinding wheel; these slides were cleaned and then coated with hydrophobic Dri-film[4] in the manner

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[2] Hereafter these nuclei, whether in the crystalline or liquid phase, will be termed "salt nuclei" or simply "nuclei."

[3] When chloride, chlorinity or Cl is used, reference is made to chlorine present as chloride or chloride ion.

[4] Dri-film No. 9987, made by the General Electric Co., Schenectady, New York.

suggested by Langmuir (1944). A Dri-film coating causes drops of water to become hemispheric on the slides, as shown in Fig. 1, where sprayed sea-water nuclei and sampled atmospheric nuclei are seen as crystalline masses and as liquid hemispheres viewed from top and side. In this study it is assumed that the nuclei, when liquid, are hemispheres. Fig. 2A shows the vane used to expose the slides normal to the air stream.

Following exposure, the slides were brought to the laboratory and placed in a lucite box which is mounted upon a microscope stage (Fig. 3). A 50 ml sample of sulfuric acid and water was introduced for vapor pressure control, after which the box was sealed. Temperature was thermostatically controlled. When the temperature of the air around the microscope and that of the acid solution inside the lucite box were equal (within $\pm$ 0.1° C), and when the drops on the slides ceased changing in diameter, it was assumed that the air in the chamber had reached water vapor saturation. The hygroscopic nuclei immersed in this air gain or lose water vapor, thus causing changes in nuclei salt concentration. At the point of equilibrium the water vapor pressure over the nuclei[5] will equal that over the acid solution, which is known. This method, which is an adaptation of a chemical technique for determining the concentration of solutions, is referred to by Glasstone (1940) as the "isopiestic" or equal pressure method.

After equilibrium was established, counts and diameter measurements of the nuclei were made. Since the vapor pressures and densities of concentrated sea water are known, it is possible to determine the weight of the chloride in the individual nuclei.

The weight, in grams, of the total chloride in all of the measured nuclei on a slide (W_{Cl}) is derived as follows:

$$W_{Cl} = V\, d\, Cl\,, \tag{1}$$

where V = volume of nuclei in μ^3, d = density of nuclei in g/cm^3 and Cl = chlorinity of nuclei expressed as weight fraction. In the equation, volume is computed from nuclei diameters; chlorinity is read from Fig. 5, using the vapor pressure and temperature of the acid solution; acid solution vapor pressures are read from Fig. 6, nuclei densities are

[5] Curvature effects upon vapor pressure over the nuclei are not generally significant when using the above method of determining the Cl present in the nuclei. This is due to the fact that the nuclei, when in equilibrium with a 16% solution of H_2SO_4 + H_2O, are hygroscopic solutions of sea salt with a concentration of Cl about 75 °/$_{oo}$ by weight. In these nuclei small increases in vapor pressure due to surface tension are readily balanced by decreases in vapor pressure produced by small increases in the salt concentration (see Fig. 5).

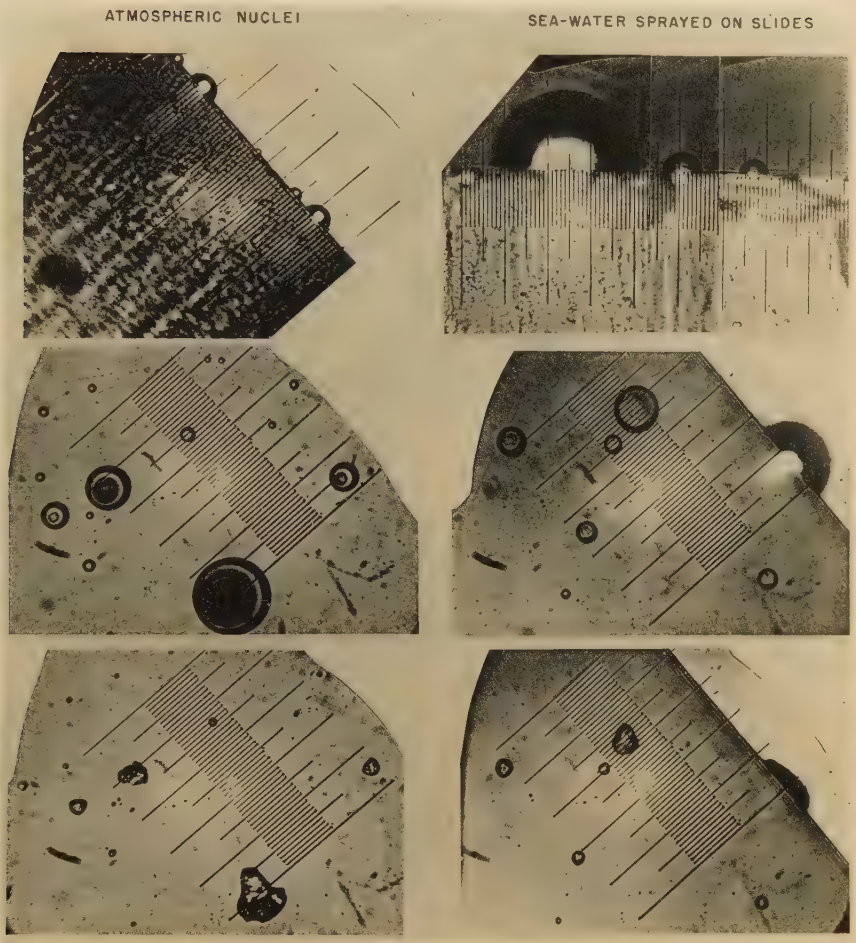

Figure 1. Photomicrographs of sea-water nuclei and sampled atmospheric nuclei as crystalline masses and as liquid hemispheres viewed from the top and side. Scale: one division = 2.2 μ

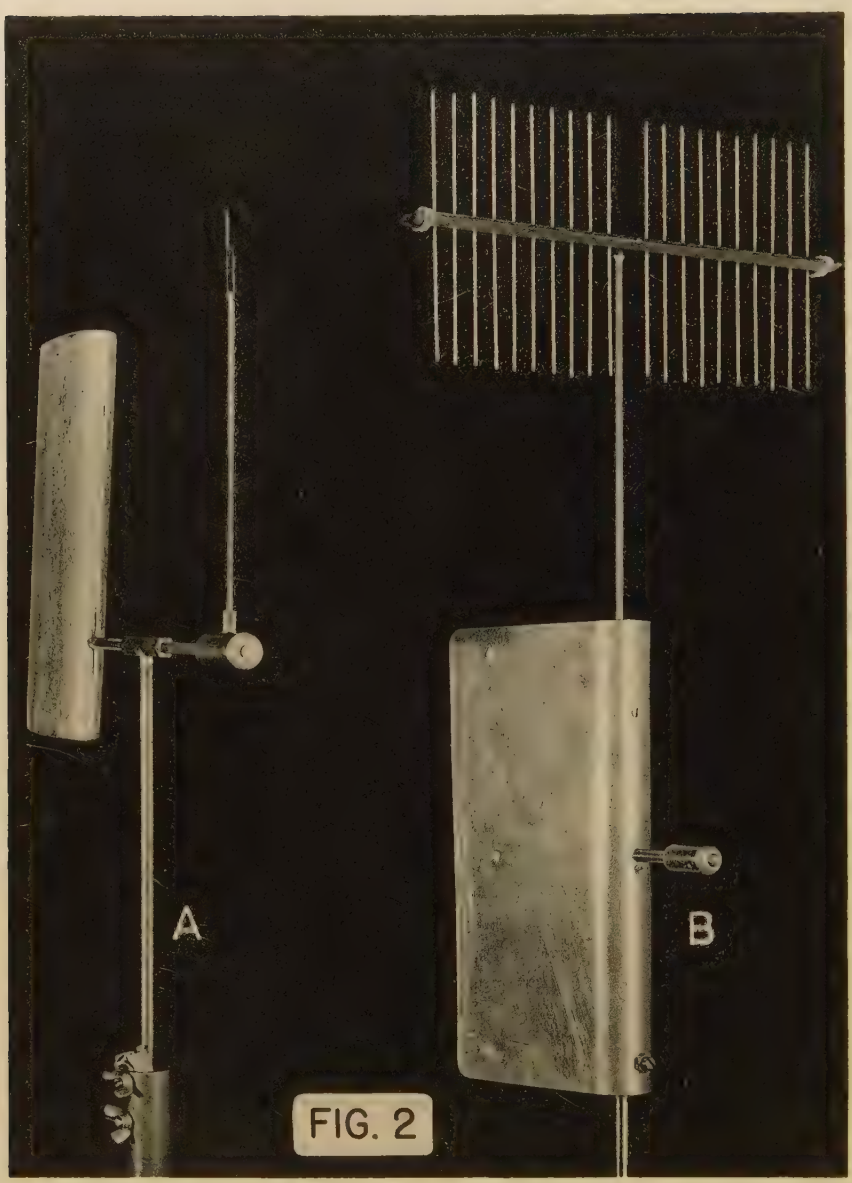

Figure 2. The glass slide and silver rod sampling surfaces and the vanes used to hold them normal to the air stream.

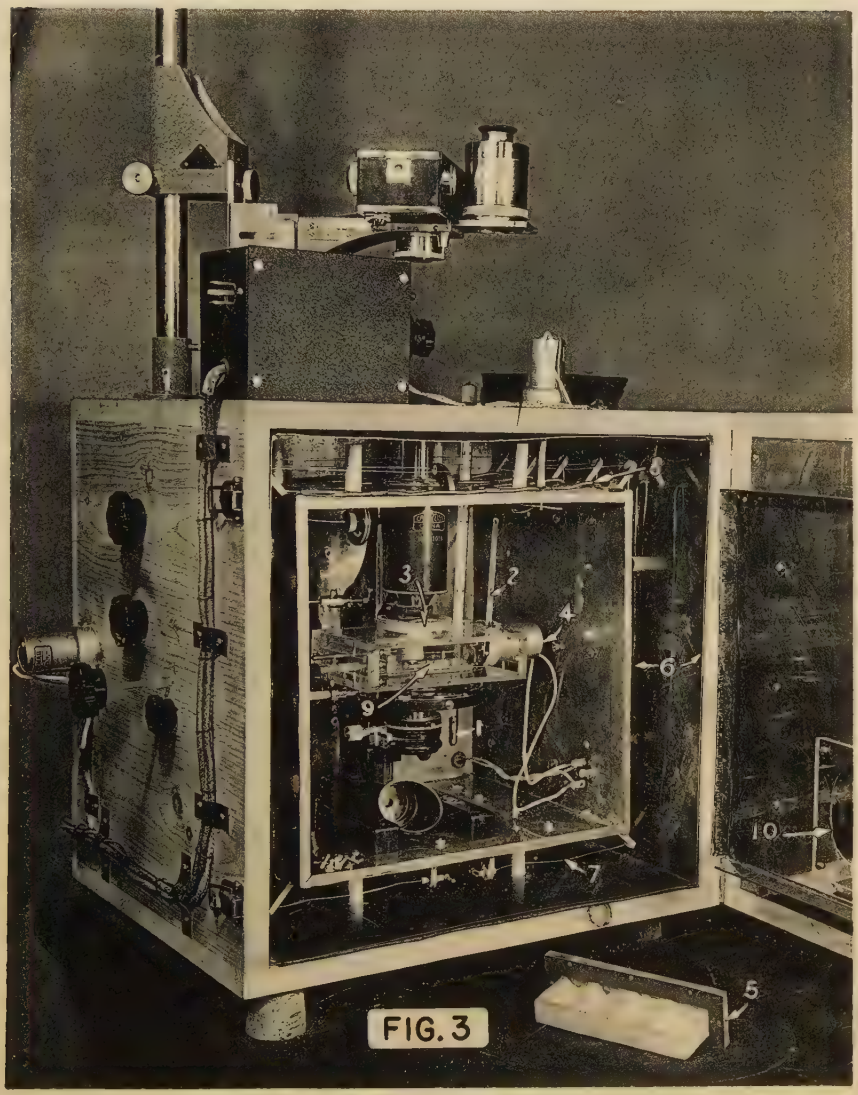

Figure 3. The lucite box and accessory apparatus used to control temperature and water vapor pressure over the nuclei. 1. Thermometer for measuring temperature of ambient air. 2. Thermometer for measuring temperature of vapor pressure control solutions. 3. Rubber diaphragm producing a flexible seal between microscope objective and lucite chamber. 4. Six volt stirring motor. 5. Container for vapor pressure control solutions which is attached to front side of lucite box. 6. Sheet copper boxes to facilitate uniform distribution of temperature. 7. Heating wires for temperature control (power source: 6 volt battery). 8. Thermometer tube of temperature control device. 9. Stage for glass slides. 10. Double lucite window in housing door for admitting light.

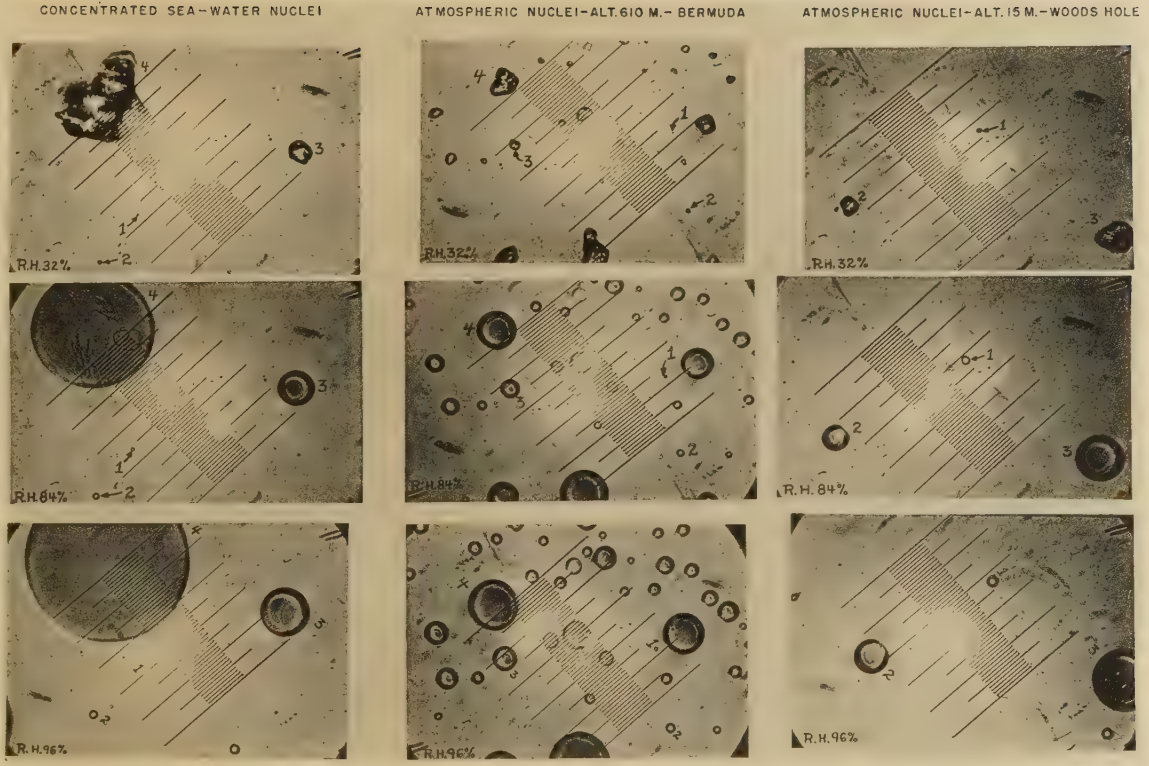

Figure 4. Photomicrographs showing changes in the appearance of nuclei as the relative humidity is increased. The nuclei at the left were sprayed on the slide.

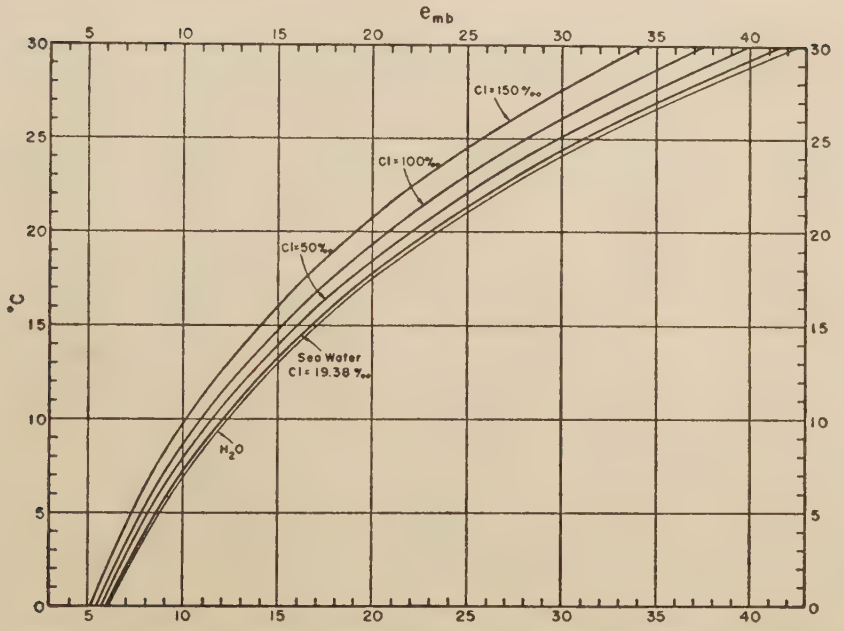

Figure 5. Saturation vapor pressure over water, sea water and concentrated sea water. Sources of data for this graph: Vapor pressure over water, from table 79 in Smithsonian Meteorological Tables; vapor pressure over sea water, from table 29 in Sverdrup, et al., 1942: 116; vapor pressure over concentrated sea water, from table 6 and chart 3 in Higashi, et al., 1931: 171, 172.

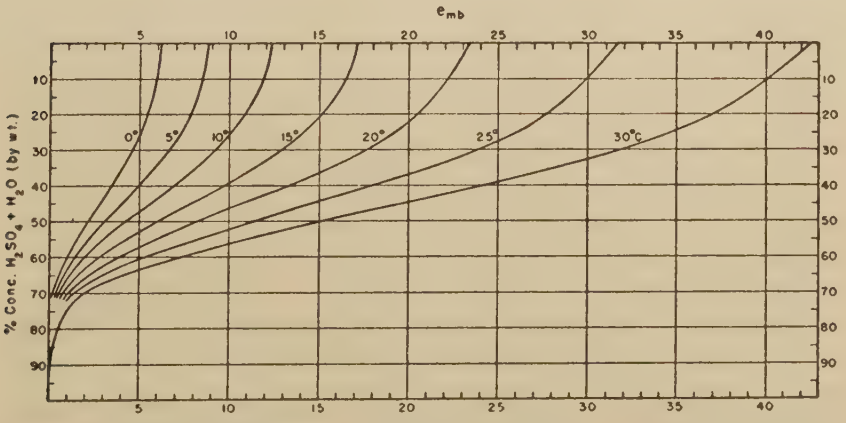

Figure 6. Water vapor pressure over solutions of $H_2SO_4 + H_2O$ (from International Critical Tables, vol. III: 302).

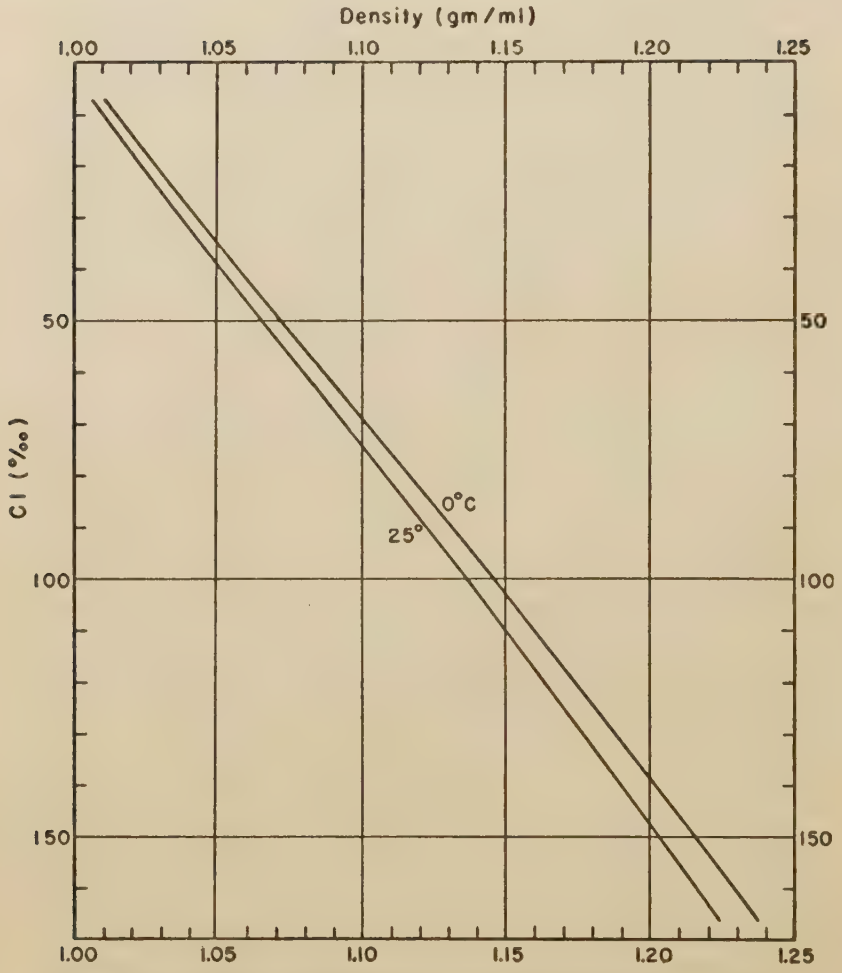

Figure 7. Density of concentrated sea water (from Higashi, et al., 1931).

read from Fig. 7. The sources of the information on these graphs are indicated below them.

I. What is the accuracy of this isopiestic method when it is used to determine the chloride content of seawater drops which are sprayed on the slides? II. Are the hygroscopic nuclei, which deposit on the slides in marine air, composed of concentrated sea water? These questions will be discussed in this order.

I. The accuracy of the isopiestic method was tested as follows. Sea water was sprayed on several slides, which were then placed in the control chamber under the microscope; the chloride content was determined over an acid solution and over sea water as well. The slides were then removed from the chamber and placed in a test tube containing water. The dissolved salts were titrated for chlorides, using the Oxner (1920) silver nitrate method.[6] Table I shows the

TABLE I. Comparison of the Nuclei *Cl* Content as Determined by Direct Titration and by a Modification of the Isopiestic Method

*No. of nuclei measured**	*Total Cl on slides by the isopiestic method*		*Cl by titration of solution of all nuclei on slides*	$\frac{C-A}{C}$	$\frac{C-B}{C}$
	A *e control with* 16% H_2SO_4 + H_2O *solution*	*B* *e control with sea water*	*C*		
160	4.1×10^{-4} gms.	———	3.5×10^{-4} gms.	−.171	———
565	2.1×10^{-4} gms.	1.5×10^{-4} gms.	2.0×10^{-4} gms.	−.050	.25
586	2.9×10^{-4} gms.	2.0×10^{-4} gms.	2.6×10^{-4} gms.	−.115	.23

* These *Cl* values are based upon diameter measurements of the nuclei deposited on about 1% of the total area over which the sea-salt nuclei were sprayed. The assumption is made that the nuclei thus measured are a representative sampling of the nuclei over the total surface.

results of three of these experiments. Computed chloride values are given in columns A and B, titrated values in column C and percent errors in the last columns. Titration values are assumed to be correct.

Thus the isopiestic method can be used to measure the chlorides in concentrated seawater drops with an accuracy of about ± 20%.

II. In regard to the chlorinity of nuclei which are sampled over the sea, the first experiment, indicating that the nuclei sampled in marine air are concentrated sea water, is qualitative in nature. Fig. 4 shows photomicrographs of nuclei at different indicated R.H. values; these pictures were selected because of the large range of drop diameters shown. The nuclei in the pictures at the left are concentrated sea water, those in the middle show nuclei sampled near Bermuda, and those on the right are of nuclei from the Woods Hole area. All nuclei are crystalline at an R.H. of 32%. At the higher R.H. values shown all nuclei are liquid, their diameters increasing with increasing R.H. In Fig. 8 are shown radii changes of the numbered nuclei in Fig. 4. The changes in radii of the atmospheric nuclei are similar to those of

[6] The accuracy of the Oxner method for titrating these small quantities of chloride was found to be ± 10%.

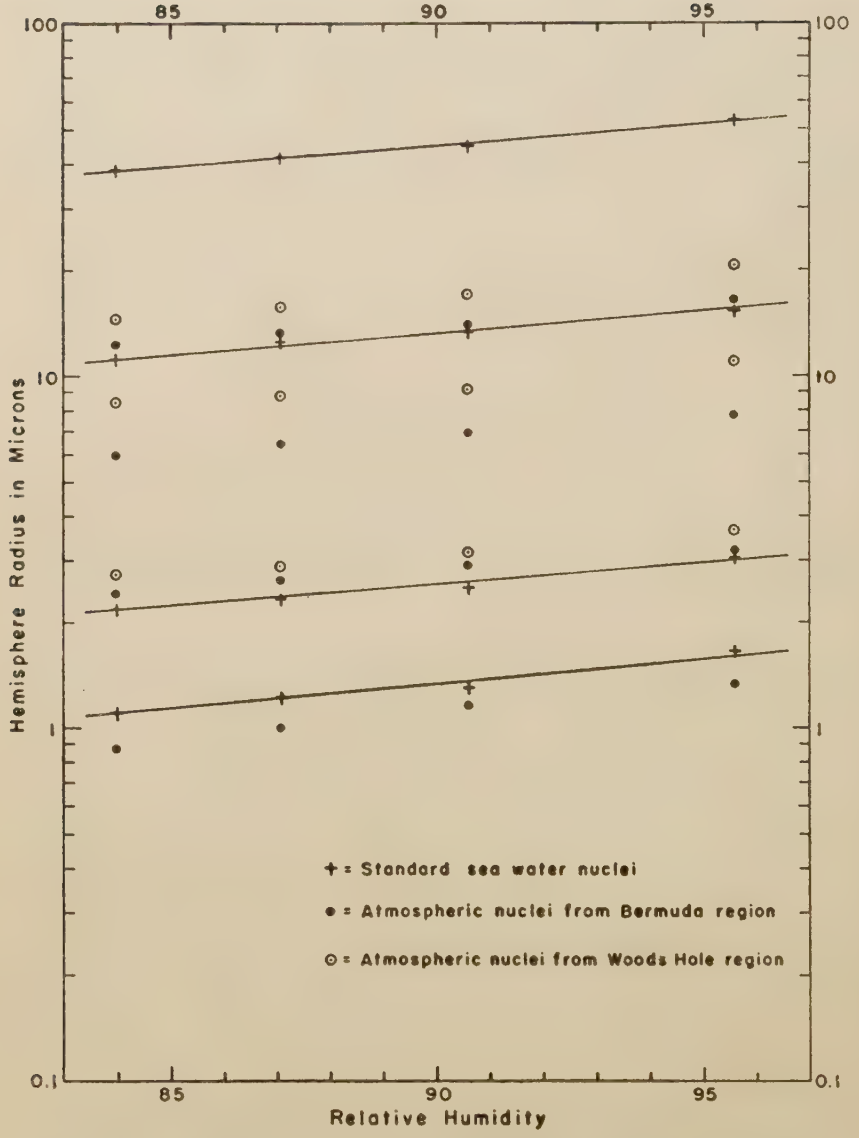

Figure 8. Changes of nuclei radii occurring as the relative humidity is increased.

the known seawater nuclei. Thus these nuclei are similarly hygroscopic and all show crystalline masses[7] at the low R.H. values.

A more quantitative method of indicating the nature of these airborne nuclei will now be described. Hard-drawn silver rods, 1 mm square, were exposed to the air at the end of a dock in Woods Hole (see Fig. 2B). These rods were usually exposed during the passage of about 100,000 meters of air, and glass slides were exposed beside the silver rods to about 2,000 meters of air. Three slide samples were taken—at the beginning, near the middle and at the end of the period of exposure of the silver rods. After exposure, the salt-encrusted silver rods were placed in water, and the dissolved salts were then titrated for chlorides. (Deposition of nuclei on the side and rear surfaces of the rods was not significant.) The samples taken on the glass slides were measured by the isopiestic method. Table II shows

TABLE II. A Comparison of the Chloride Content of the Air, as Determined by the Isopiestic and Titration Methods. Sampling Height 4 Meters over the Sea Surface. Location: the Seaward End of the Marine Biological Laboratory Dock, Woods Hole, Massachusetts

Date	*Wind Velocity*	*Total nuclei measured on three slides*	R_l*	*A Av. Cl by isopiestic method (g/cm³ of air)*	*B Cl by titration of a solution of nuclei from silver rods (g/cm³ of air)*	$\frac{B-A}{B}$	R_{Cl}†
VI.14.48	4.9 m/s SW	458	.06	0.54×10^{-12}	1.05×10^{-12}	+.49	.46
VI.17.48	6.3 m/s SW	883	.05	1.17×10^{-12}	1.51×10^{-12}	+.23	.42
VI.18.48	5.3 m/s SSW	426	.03	0.55×10^{-12}	0.68×10^{-12}	+.28	.14
VI.30.48	7.6 m/s SW	1532	.03	2.73×10^{-12}	2.72×10^{-12}	0	.80

* R_l = Ratio of total exposure length of three glass slides to exposure length of silver rods.

† R_{Cl} = Ratio of minimum value to maximum value for atmospheric chlorides measured by the glass-slide isopiestic method.

the results of these measurements; column A gives the average chloride computed from the drops on the three slides, column B the values for chloride in the nuclei deposited upon the silver rods, the next to last column the percent error of the glass-slide isopiestic method, assuming

[7] It may be supposed that crystalline nuclei will fail to adhere to the glass slides upon impact, thus producing unknown errors in this method of sampling. It is probable, however, that the presence of the highly soluble magnesium and calcium chlorides in sea salt will cause crystalline material of lower solubility in a nucleus to remain wet at relative humidities as low as 33% (see International Critical Tables, Vol. 3: 367, 368 for vapor pressures over saturated aqueous solutions of $MgCl_2$ and $CaCl_2$). For this reason it is assumed here that crystalline sea-salt nuclei, at relative humidities above about 30%, will adhere to the glass slides upon impact as readily as they do when completely liquified.

that the titration values are correct. The difference between the two methods is not great in consideration of the small quantities measured and of the rather large maximum variations of chloride shown by the individual slides (see column R_{Cl}). The largest percent error, 0.49, is attributed to these variations within the air stream. (The relatively short exposure lengths of the slides, indicated by column R_l, were used in order to measure the size distribution of the nuclei, as it was not thought at the time that there would be so much variability of salt content within the air stream.)

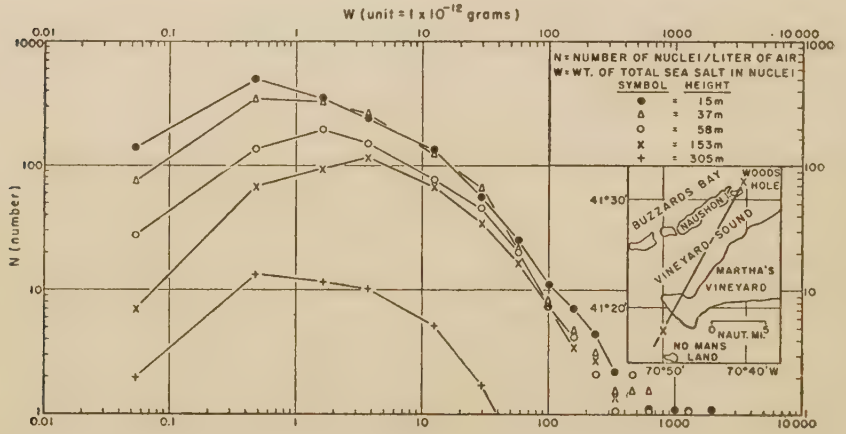

Figure 9. The numbers and weights of sea-salt nuclei sampled in the lower atmosphere over the sea (see Table III for position, time, etc.).

Thus the isopiestic method indicates a chlorinity of atmospheric nuclei approximately the same as the chlorinities derived by titration of deposited salts. The method also indicates that the atmospheric nuclei are similarly hygroscopic when compared to concentrated seawater nuclei, and show similar crystalline masses at low R.H. values. Therefore, the isopiestic method seems useful in measuring the weights and numbers of atmospheric nuclei which are sampled.

Fig. 9 shows the numbers and weights of nuclei that were sampled from an aircraft in air which had passed over about 150 miles of sea surface. The lines are added to aid the eye in following the trend of the observed points. The weights of the nuclei represent the total sea salts present, assuming that the total salts are equal to 1.805 × chlorinity, expressed as parts per thousand by weight. Each set of symbols represents the weight and number distribution at some specified height. (The weight of the smallest nucleus shown is based

upon an estimated drop diameter.) The "X" mark on the insert chart shows the location of the sampling area.

Fig. 10 shows the data in Fig. 9 corrected for deposition error; that is, correction has been made for the fact that some of the air-borne

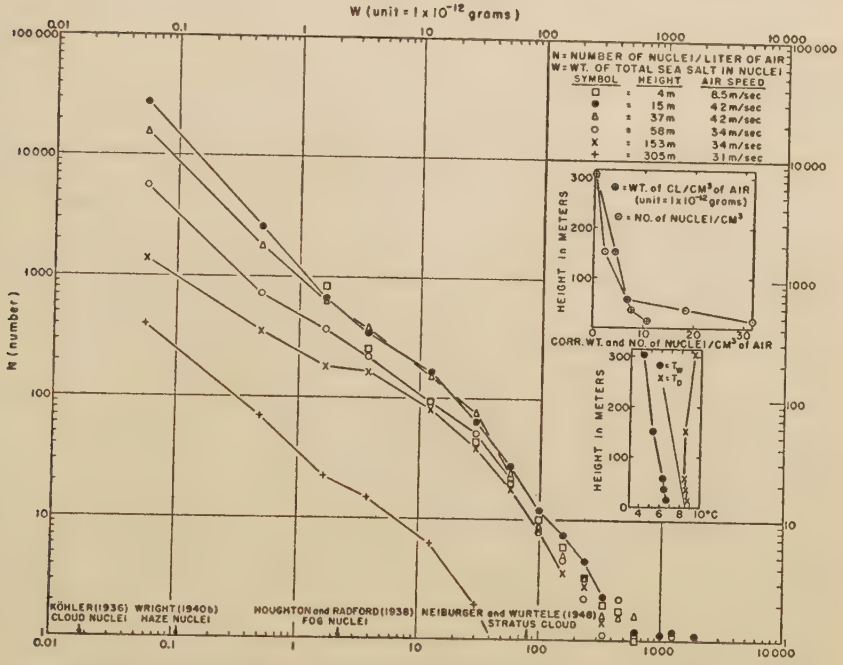
W (unit = 1 x 10⁻¹² grams)
N (number)
N = NUMBER OF NUCLEI / LITER OF AIR
W = WT. OF TOTAL SEA SALT IN NUCLEI
SYMBOL HEIGHT AIR SPEED
4 m 8.5 m/sec
15 m 42 m/sec
37 m 42 m/sec
58 m 34 m/sec
153 m 34 m/sec
305 m 31 m/sec
HEIGHT in METERS
● = WT. of CL/CM³ of AIR (unit = 1 x 10⁻¹² grams)
⊙ = NO. of NUCLEI/CM³
CORR. WT. and NO. of NUCLEI/CM³ of AIR
● = T_w
X = T_D
10°C
KÖHLER (1936) CLOUD NUCLEI
WRIGHT (1940b) HAZE NUCLEI
HOUGHTON and RADFORD (1938) FOG NUCLEI
NEIBURGER and WURTELE (1948) STRATUS CLOUD

Figure 10. The data in Fig. 9 corrected for deposition error (E). The square symbols represent the corrected deposition of nuclei on two slides exposed at wind speed before and after the airplane samples were taken. Above the lower *W* scale are shown the average or mean values for sea-salt nuclei weight as derived by various authors.

nuclei are deflected by the flow of air around the slides and hence fail to impinge upon them, This error, for the very small nuclei, is large, and there is some uncertainty as to its magnitude for the smallest nucleus shown. The inserts in Fig. 10 show variations with height of air temperature and of nuclei weight and number. Table III shows other information concerning the data in Figs. 9 and 10.

For correction of deposition error, reference was made to a mathematical study by Langmuir and Blodgett (1945) on water droplet trajectories and deposition. They found the maximum total efficiency of drop deposition on ribbons, assuming ideal fluid flow. Drop

TABLE III. Additional Data Relative to Atmospheric Nuclei Measurements Shown in Figs. 9 and 10. Date, April 30, 1948. Position, Latitude 41° 18′ N., Longitude 70° 50′ W. Surface Wind, Force 4, Direction SW. Probable Fetch, < 200 Nautical Miles. Atmospheric Pressure 1002 to 1006 mb. Sky, Clear

1	2	3	4	5	6	7	8		9		10		11
Time (EDT)	*Height (m.)*	*Air speed (m/sec)*	T_D*	T_W*	e_{mb}	*R.H.*	*Cl in g/cm³ of air. Unit = 1×10^{-12}*		*No. of nuclei per cm³ of air*		*Covering fraction*		*No. of*
							uncor.	*cor.*	*uncor.*	*cor.*	*nuclei cryst.*	*nuclei Cl conc. 7%*	*nuclei counted*
1350†	4	9	9.9	8.8	10.5	86	2.98	5.01	0.16	1.2	——	0.009	386
1500	15	42	8.8	6.7	8.4	74	9.07	10.93	1.48	31.9	0.004	0.023	662
1508	37	42	8.7	6.5	8.2	73	6.1	7.77	1.25	18.6	0.005	0.029	795
1515	58	34	8.5	6.4	8.2	73	5.43	6.82	0.68	7.1	0.004	0.025	635
1520	153	34	8.5	5.3	6.9	62	3.56	4.44	0.43	2.3	0.004	0.024	612
1528	305	31	9.4	4.3	5.0	42	0.12	0.23	0.045	0.5	0.001	0.003	516
1740†	4	8	9.5	8.7	10.7	90	5.92	8.3	0.19	1.4	——	0.013	456

* All T_D and T_W measurements made from aircraft were read from sling psychrometer thermometers held in the air stream. Correction for temperature rise due to dynamic heating on the dry bulb (ΔT_d) and the wet bulb (ΔT_w) is approximated (from Spilhaus, 1943) as follows:

$$\Delta T_d = a_d \left(\frac{V}{100}\right)^2 ,$$

and

$$\Delta T_w = a_w \gamma \left(\frac{V}{100}\right)^2 ,$$

where a_d and a_w depend upon the form of the thermometers and γ is the ratio of the wet to the dry lapse rates. A value of 0.85 is used for both a_d and a_w.

† At this time slides were exposed at the end of a dock in Woods Hole. These samples were taken before and after the flight on which the other samples were obtained.

deposition efficiencies (E) are given in terms of two dimensionless parameters K and Φ, which are defined in the following equation:

$$K = \frac{2\rho s\, a^2 U}{9\eta C}, \tag{2}$$

and

$$\Phi = \frac{18\rho a^2\, CU}{\eta \rho s},$$

where a = drop radius in cm, ρa = air density in g/cm^3, ρs = drop density in g/cm^3, η = viscosity of air in poises (g/cm sec), C = ½ width of slide in cm, and U = wind speed in cm/sec.

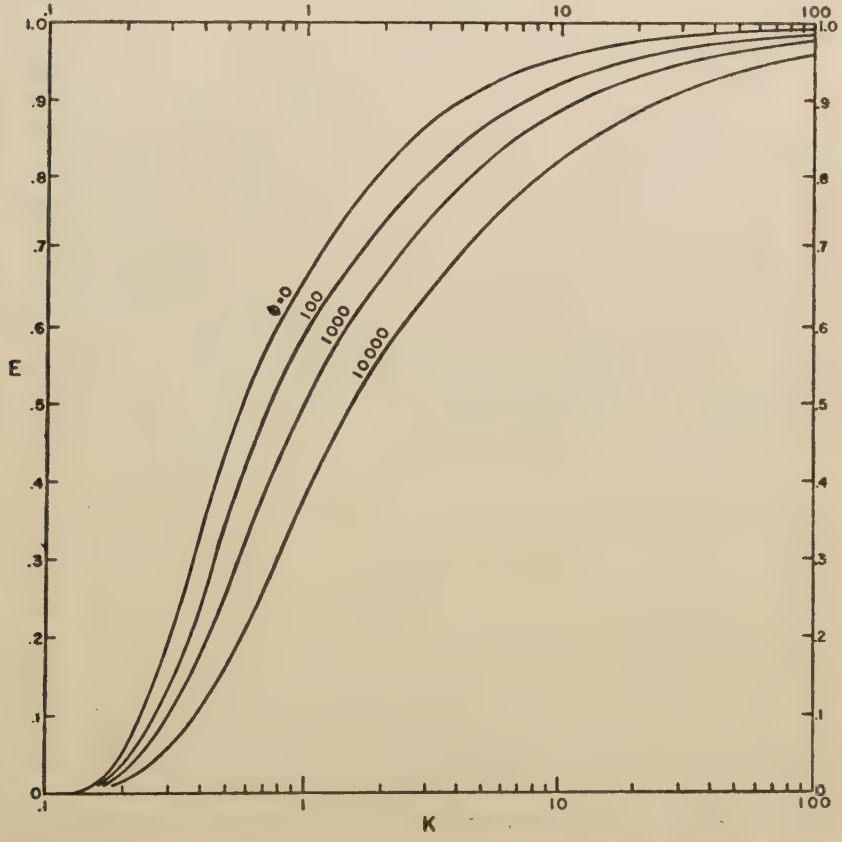

Figure 11. Efficiency of rime deposition on ribbons with ideal fluid flow (from Langmuir and Blodgett, 1945).

Constant values for drop density, air density, air viscosity and width of ribbon were substituted in these equations, leaving drop radius and wind speed as the two variables. Wind speed was fixed arbitrarily, while Φ and K were calculated for drop radii varying from 0.3 to 14 microns. Deposition error (E) was then taken from the results of Langmuir and Blodgett (see Fig. 11), using the Φ and K values

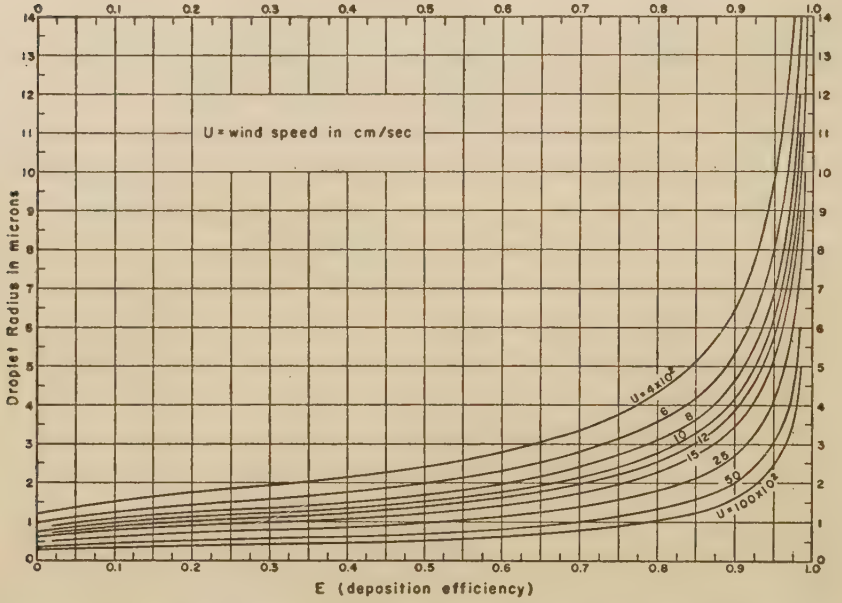

Figure 12. Deposition efficiency of water droplets upon "ribbons" one millimeter wide (Langmuir and Blodgett, 1945).

derived from the equations. This process was carried out for wind speeds ranging from 4 to 100 m/sec. The results are plotted in Fig. 12, which can be used in deriving E for nuclei samples taken in an air temperature range of 0° to 25° C., with a maximum error in E of 3%.

A nuclei density of one was used in deriving the E values plotted in Fig. 12. To avoid separate calculations and curves for the deposition efficiency of nuclei of varying densities, a simple correction may be applied to Fig. 12 for those cases where nuclei density is significantly different from one. The equations for K and Φ, and the family of curves for K, Φ and E (see Fig. 11), indicate that proportionate changes of density or wind speed affect the resulting computed value

of E similarly. Thus, doubling the value of ρ_s gives about the same value of E as does the doubling of U. As a specific example of the use of Fig. 12, assume that nuclei of 1 μ radius are sampled at an air speed of 30 m/sec and at a time when their density in the free air is 1.2 [see equation (3) for the method used to derive nuclei radii in the free air]. In this case the modified value for air speed is 1.2 $\times$ 30, or 36 m/sec. A nucleus of 1 μ radius which was sampled on a 1 mm wide ribbon and at a modified air speed of 36 m/sec has a deposition efficiency of about 0.6 (see Fig. 12).[8]

The radius (R) of the nuclei in the free air at the time of sampling is derived as follows:

$$R = \sqrt[3]{\frac{W}{\frac{c\,D}{4/3\pi}}} \quad . \tag{3}$$

W = the weight of chloride in the nucleus (unit = 1×10^{-12} g). c = nucleus chloride concentration by weight fraction; taken from Fig. 3, using the dry-bulb temperature and the vapor pressure of the free air where the samples were taken.[9] D = nucleus density in g/cm^3; taken from Fig. 6, using the dry-bulb temperature of the air in which the samples were taken and the nucleus concentration derived above.

It has been necessary to discuss deposition error at this time in order to point out the errors of impact sampling.

Are nuclei of the weights and numbers which were sampled at air speeds up to 42 m/sec actually present in the air? Is it not possible that coalescence or shattering may have altered the nuclei weight and number distribution?

Langmuir (1944) has shown that coalescence of cloud and fog drops, upon impact on glass slides, may modify significantly the size distribution when the covering fraction (i. e., the per cent of the slide surface covered by the drops) exceeds 0.1. A direct test for possible coales-

[8] For the ranges of sampling speed, nuclei radii and nuclei density discussed in this paper, the above method of allowing for the variation of nuclei density produces a value of E which is correct to within 3%.

[9] In those cases where the free air humidity is between 78 and 70%, chloride concentration is derived by extrapolation of the results of Higashi et al. (1931) (Fig. 3). Below a humidity of 70% it is assumed that the nuclei are mostly crystalline (from Owens, 1926) with a density of about 2.2. In deriving deposition error E it is also assumed that these crystalline nuclei deposit on the slides as would spherical droplets of the same density (see footnote 7).

cence and shattering was made at an R.H. of 82%, when the nuclei are always liquid.

Six glass slides were exposed at constant altitude over the sea—three at higher and three at lower air speeds. Fig. 13 shows the distribution of weights and numbers of the nuclei sampled. It can be

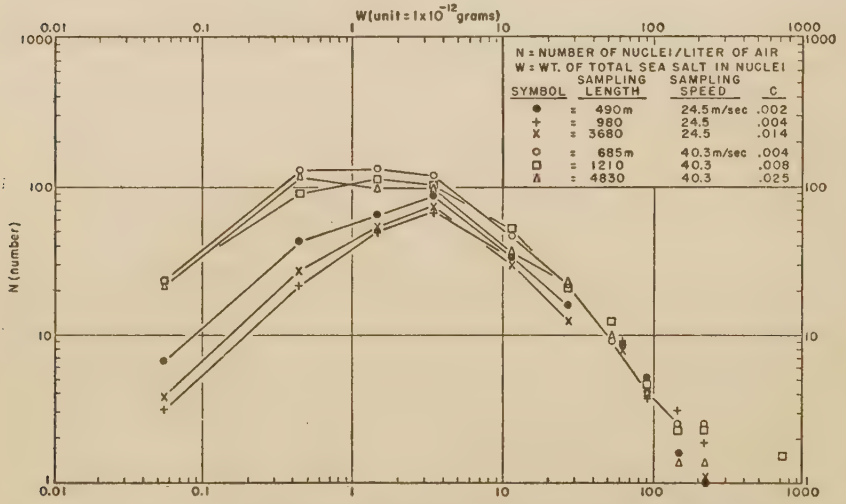

Figure 13. Variations of nuclei weight and number with different sampling lengths and speeds (see Table IV for further data).

seen on this slide that there is little difference in the numbers and weights of nuclei sampled on three slides of increasing covering fraction (C) exposed at a speed of 24.5 m/sec (see the lower plotted symbols). Similarly, Fig. 13 shows little difference in the number and weight of nuclei sampled at an air speed of 40.3 m/sec.

If a significant amount of coalescence of nuclei had occurred at constant air speed, Fig. 13 should show an increasing proportion of larger nuclei and a decreasing proportion of smaller nuclei associated with increasing values of C. Fig. 13 shows no trend towards larger nuclei which is related to increasing covering fraction. It is concluded as improbable, therefore, that coalescence of nuclei was a significant factor in producing the weight range shown.

Do the nuclei become shattered by impact? Fig. 13 indicates an increase in the numbers of nuclei sampled with a speed increase. Differences in deposition efficiency account for much of this discrepancy in number. In Fig. 14 are plotted average N values (from

Fig. 13) to which the deposition error correction has been added. The remaining difference in the average N at the two sampling speeds is attributed to changes, between sampling periods, in the amount of chlorides present in the air, as shown in Table IV. Note that the

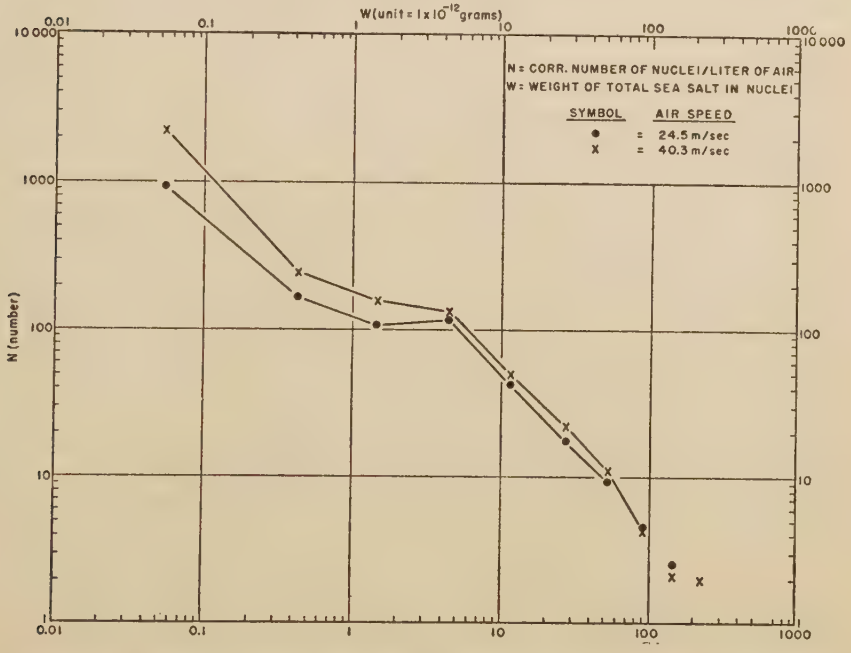

Figure 14. Average N values, from Fig. 13, which have been corrected for deposition error.

largest values in column 8, corrected and uncorrected, correspond to the higher air speed (column 2). This change can be due to variations of N with time or to slight variations in sampling height.

Shattering tests have also been made when nuclei are crystalline, at an R.H. of 43%. No shattering has been observed.

The sampling results which have been shown and discussed indicate that neither coalescence nor shattering of nuclei upon impact is a significant factor in the production of the nuclei weight and number distributions shown. This conclusion gains further support from the similarity of the corrected deposition of nuclei on slides exposed at an average wind speed of 8.5 m/sec and at 42 m/sec (see Fig. 10). The square symbols represent samples taken near the surface at Woods

TABLE IV. Data Relative to a Test of the Effects of Air Speed and Exposure Length Upon the Weight and Number Distribution of Nuclei Deposited on Glass Slides (see Fig. 13). Date, June 25, 1948. Position, Latitude 41° 18′ N., Longitude 70° 50′ W. Surface Wind, Force 3, SW. Atmospheric Pressure, 1006 mb. Time, 1700 to 1720 EDT

1 *Height* (*m*)	2 *Air speed*	3 *Slide exposure length in:*		4 T_D	5 T_W	6 e_{mb}	7 *R.H.*	8 *Cl per cm³ of air. Unit* = 1×10^{-12} *g*		9 *No. of nuclei per cm³ of air uncorrected*	10 *Covering fraction* (%)	11 *No. of nuclei counted*
		sec	*m*					*uncor.*	*cor.*			
15	24.5	20	490	18.75	16.75	17.6	82	2.92	3.48	.26	.002	517
15	24.5	40	980	——	——	——	——	3.14	3.60	.21	.004	558
15	24.5	150	3680	——	——	——	——	3.16	3.62	.22	.014	581
15	40.3	17	685	——	——	——	——	4.42	5.00	.49	.004	597
15	40.3	30	1210	——	——	——	——	6.66	7.26	.43	.008	563
15	40.3	120	4830	——	——	——	——	4.25	4.74	.41	.025	608
61	24.5	——	——	23.35	18.05	17.0	60	——	——	——	——	——
2*	4	60	240	17.9	17.5	19.5	95	78.0	——	——	——	544

* These measurements were made on a beach two nautical miles downwind from the sea position given above. Nuclei produced by the local surf probably account in large part for the increase in *Cl* per cm³ of air as shown by this beach sample (see column 8).

Hole after the lower air had been mixed in passing over small islands (see insert, Fig. 9). The close agreement is evident. Sea fog nuclei, sampled at 7 m/sec, also show a closely similar distribution in the weight range of 5×10^{-12} g and less, again indicating the presence of the smaller salt nuclei in the sea air. (Sea fog nuclei samples from the Woods Hole area have indicated thus far that sea salt is the nucleus upon which fog drops form.)

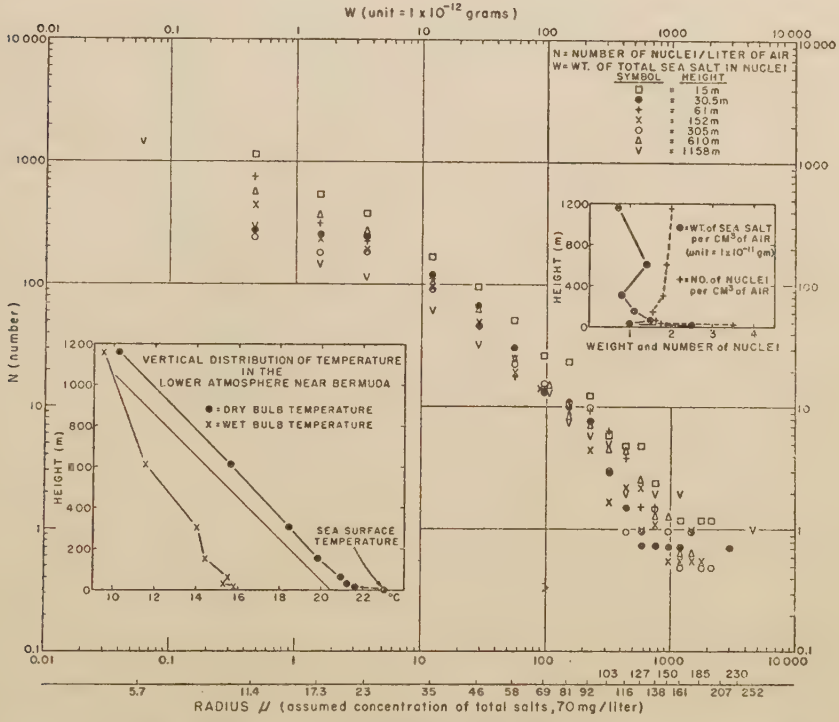
W (unit = 1 x 10⁻¹² grams)
N = NUMBER OF NUCLEI / LITER OF AIR
W = WT. OF TOTAL SEA SALT IN NUCLEI
SYMBOL HEIGHT
15 m
30.5m
61 m
152m
305m
610m
1158m
N (number)
HEIGHT (m)
VERTICAL DISTRIBUTION OF TEMPERATURE IN THE LOWER ATMOSPHERE NEAR BERMUDA
● = DRY BULB TEMPERATURE
X = WET BULB TEMPERATURE
SEA SURFACE TEMPERATURE
°C
● = WT. of SEA SALT per CM³ of AIR (unit = 1 x 10⁻¹¹ gm)
+ = NO. of NUCLEI per CM³ of AIR
WEIGHT and NUMBER of NUCLEI
RADIUS μ (assumed concentration of total salts, 70 mg/liter)

Figure 15. The numbers and weights of sea-salt nuclei sampled in the lower atmosphere over the sea in the Bermuda area (see Table V for additional information).

Finally, Fig. 15 shows the corrected weight and number of nuclei sampled near Bermuda in air which had passed over at least 600 miles of sea surface. Table V shows further data from this area.

It is interesting to compare the vertical distribution of weight and number of nuclei in the mixed air near Bermuda with the vertical distribution of weight and number of nuclei in thermally stable air near Woods Hole (Fig. 10). In the thermally stable air the total

TABLE V. Data Concerning Air-borne Sea-salt Samples Taken Near Bermuda on November 26, 1948. Position, Latitude 32° 23′ N. Longitude 64° 55′ W. Surface Wind N, Force 3 to 4. Clouds 2 cu.

1	2	3	4	5	6 T†		7	8	9		10		11	12
Time (zone 5)	*Height* (*m*)	*Slide exposure* (*secs*)	*Atmos-pheric pressure*‡ (*mb*)	*Air speed*ø (*m/sec*)	T_d	T_w	e_{mb}	*R.H.*	*Sea salt in g/cm³ of air; unit = 1 × 10⁻¹²*		*No. of nuclei per cm³ of air*		*Covering fraction* (%)	*No. of nuclei measured*
									uncor.	*cor.*	*uncor.*	*cor.*		
1535	15.2	30	1016.5	31.8	21.6	15.75	10.80	42	30.6	34.6	.70	2.5	.004	738
1540	30.5	60	1015	26.8	21.2	15.30	10.43	42	15.1	17.2	.409	1.0	.004	580
1546	61	60	1012	26.8	20.9	15.5	11.23	45	14.1	16.3	.407	1.5	.004	552
1555	152	120	1000	26.8	19.8	14.4	10.42	45	13.5	15.3	.34	1.1	.006	640
1603	305	120	983	26.8	18.4	14.0	11.45	54	16.0	18.0	.313	0.8	.007	670
1612	610	120	946	26.8	15.6	11.5	10.28	58	16.8	19.3	.44	1.4	.008	723
									*19.8	20.1	.54	.7		
1620	1158	90	886	31.3	10.2	9.5	11.46	92	19.8	20.2	.69	2.1	.093	667

‡ Sea level pressure (1018 mb) was recorded at the Meteorological Office in Hamilton, Bermuda, at 1600. Pressures at other heights are based upon the U. S standard atmosphere.

ø Altitude correction of indicated air speed was not applied. At a height of 1158 m this correction amounts to about 2.1 m/sec. At lower altitudes it is not significant.

† T_d and T_w values are corrected for dynamic heating of thermometers.

* The upper of the two values in columns 9 and 10 (alt. 1158 m.) does not include the smallest nuclei sampled at this height. These upper figures should be used for comparison with values obtained at lower altitudes, since the range of nuclei weight is the same. The lower value includes the smallest nuclei sampled. The deposition of smaller nuclei at the 1158 m. height (see Fig. 15) results from an increase in deposition efficiency with increasing nuclei radii associated with higher R.H. values.

number and total weight of sea-salt nuclei decreased rapidly with height. Near Bermuda, in well mixed air overlying warmer water, there is relatively little change with altitude in nuclei number and weight (see inserts, Fig. 15). This difference in vertical distribution of nuclei is probably a result of the difference in vertical mixing in the two air masses concerned.

At the base of Fig. 15 a second scale is drawn showing the computed radii of the nuclei, assuming spherical drops with a concentration of sea salt of 70 mg/l (7×10^{-17} g/μ^3). In each case the radius indicated is that of a nucleus containing a weight of sea salt equal to the weight of the sea salt in nuclei actually sampled. The concentration of 70 mg/l is the average value given by Houghton and Radford (1938: 30) for the total salt content of fog water at Round Hill, Mass. This concentration is much greater than the average of the figures given by Wright (1940a) in his summary of the measurements which have been made of the chloride concentration of snow, rime, rain and cloud waters. Therefore, a concentration of 70 mg/l seems a conservative figure for salt concentrations in marine cloud drops. The computed drop sizes, based upon this concentration, are therefore assumed to be a reasonable approximation of the eventual cloud drop sizes resulting from the lifting of the air containing these nuclei. Cloud drops having the radii shown at the base of Fig. 15 will have rates of fall varying from about 0.3 to 200 cm/sec (see Humphreys, 1940: 280). Drops of the larger sizes (radius 50 μ or more) are classified as mist, drizzle or light rain by Humphreys.

Thus this great range of nuclei weight at the base of an oceanic cloud, if it proves to obtain generally over the seas, may explain the occurrence of rain from thin layers of stratus clouds ($T > 0°$ C.) in oceanic areas (Kotsch, 1947; Schaefer, 1949).

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SUMMARY

A method of sampling air-borne sea-salt nuclei over the open ocean is described. Data are given showing the distribution of the weight and number of nuclei sampled at various heights. It is suggested that these sea-salt nuclei act as condensation nuclei in the formation of sea fogs and clouds, and that the presence of the larger nuclei at cloud bases may account for the production of rain from small clouds in marine locations at temperatures above 0° C. The range of the

weights of these nuclei is comparable to the range of the average weights of salt nuclei which have been measured at shore stations by other authors.

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